



**LIQUID-PHASE BIOREACTOR FOR DEGRADATION
OF TRICHLOROETHYLENE AND BENZENE**

Bill Guarini and Brian Folsom

**Envirogen, Inc.
4100 Quakerbridge Road
Lawrenceville NJ 08648**

**ENVIRONICS DIRECTORATE
139 Barnes Drive, Suite 2
Tyndall AFB FL 32403-5323**

April 1996

Final Technical Report for Period January 1993 - December 1994

19960913 138

DISTRIBUTION LIMITED TO U.S. GOVERNMENT AGENCIES ONLY BECAUSE OF PROPRIETARY NATURE OF RESEARCH. DISTRIBUTION LIMITATION APPLIED JANUARY 1995; WILL BE IN EFFECT FOR 5 YEARS. OTHER REQUESTS FOR THIS DOCUMENT MUST BE REFERRED TO AL/EQPP, 139 BARNES DRIVE, SUITE 2, TYNDALL AIR FORCE BASE, FLORIDA 32403-5323.

Handling and Destroying Unclassified/Limited Distribution Documents - Unclassified/Limited Distribution documents shall be handled using the same standard as "For Official Use Only (FOUO)" material, and will be destroyed by any method that will prevent disclosure of contents or reconstruction of the document. When local circumstances or experience indicates that this destruction method is not sufficiently protective of unclassified limited information, local authorities may prescribe other methods but must give due consideration to the additional expense balanced against the degree of sensitivity.

**AIR FORCE MATERIEL COMMAND
TYNDALL AIR FORCE BASE, FLORIDA 32403-5323**

FOR OFFICIAL USE ONLY

**ARMSTRONG
LABORATORY**

NOTICES

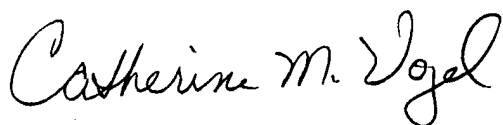
This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any employees, nor any of their contractors, subcontractors, nor their employees, make any warranty, expressed or implied, or assume any legal liability or responsibility for the accuracy, completeness, or usefulness or any privately owned rights. Reference herein to any specific commercial process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency, contractor, or subcontractor thereof. The views and opinions of the authors expressed herein do not necessarily state or reflect those of the United States Government or any agency, contractor, or subcontractor thereof.

When Government drawings, specifications, or other data are used for any purpose other than in connection with a definitely Government-related procurement, the United States Government incurs no responsibility or any obligations, whatsoever. The fact that the Government may have formulated or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication, or otherwise in any manner construed, as licensing the holder or any other person or corporation; or as conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Distribution authorized to U. S. Government agencies only because of the proprietary nature of the research. Limitation applied January 1995 and will be in effect for 5 years. Other requests for this document must be referred to AL/EQPP, 139 Barnes Drive, Suite 2, Tyndall Air Force Base, Florida, 32403-5323.

This report has been reviewed and is approved for publication.

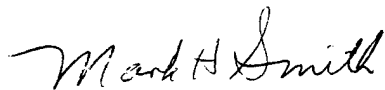
DESTRUCTION NOTICE: DESTROY BY ANY METHOD THAT WILL PREVENT DISCLOSURE OF CONTENTS OR RECONSTRUCTION OF THE DOCUMENT.



CATHERINE M. VOGEL, MS
Bioremediation Technical Area Manager



MICHAEL G. KATONA, PhD
Chief Scientist, Environics Directorate



MARK H. SMITH, Major, USAF, BSC
Chief, Site Remediation



NEIL J. LAMB, Colonel, USAF, BSC
Director, Environics Directorate

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.				
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE April 1996	3. REPORT TYPE AND DATES COVERED Final Report 11 Jan 93 - 30 Dec 1994		
4. TITLE AND SUBTITLE Liquid-Phase Bioreactor for Degradation of Trichloroethylene and Benzene		5. FUNDING NUMBERS		
6. AUTHOR(S) Bill Guarini and Brian Folsom		8. PERFORMING ORGANIZATION REPORT NUMBER		
7. PERFORMING ORGANIZATION NAMES(S) AND ADDRESS(ES) Envirogen, Inc. 4100 Quakerbridge Road Lawrenceville, NJ 08648		10. SPONSORING/MONITORING AGENCY REPORT NUMBER Final Report AL/EQ 1995-0001		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) AL/EQW-OL 139 Banres Drive, Suite 2 Tyndall AFB FL 32403-5323		11. SUPPLEMENTARY NOTES This is a Phase II SBIR final report. Availability of this report is specified on reverse of front cover. AL/EQW Project Officer: Catherine M. Vogel (904) 283-6208; DSN 523-XXXX		
12a. DISTRIBUTION/AVAILABILITY STATEMENT Distribution authorized to US Government agencies only because of proprietary nature of research. Limitation applied January 1995; will be in effect for 5 years. Other requests for this document must be referred to AL/EQPP, 139 Barnes Dr, Suite 2, Tyndall AFB FL 32403.		12b. DISTRIBUTION CODE B		
13. ABSTRACT (Maximum 200 words) The major objective was to demonstrate the efficacy of a dual-stage bioreactor system for the treatment of groundwater contaminated with fuels and solvents. Under this SBIR Phase II, the number of chemicals to be treated during the field demonstration was expanded to include TCE, BTEX, and dichlorobenzenes (DCBs). A pilot-scale dual-stage bioreactor system was operated at Robins AFB GA to establish proof-of-concept and to develop operational and economic information for full-scale implementation. The system included a fluidized-bed reactor (FBR), an air stripper, and a TCE gas-phase bioreactor (GPR). Due to the enhanced performance of the FBR towards TCE, the demonstration essentially became a test of two independent pilot systems, one for treatment of contaminated water in the FBR and the second for treatment of contaminated air in the GPR. Operation of the system demonstrated effective treatment of not only BTEX and DCB, but also TCE. Over 210,000 gallons of contaminated groundwater were effectively treated during the demonstration. All hazardous chemicals were treated to concentrations near or below drinking water standards. An economic evaluation of the FBR to UV-peroxidation, air stripping with carbon adsorption, wet carbon adsorption and air stripping followed by PURUS adsorption suggests a significant cost savings over the life of a typical project.				
14. SUBJECT TERMS Dual-stage bioreactor, trichloroethylene (TCE), BTEX, dichlorobenzenes, fluidized-bed reactor (FBR), gas-phase bioreactor (GPR),			15. NUMBER OF PAGES 67	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF REPORT Unclassified	19. SECURITY CLASSIFICATION OF REPORT Unclassified	20. LIMITATION OF ABSTRACT UL	

PREFACE

This report was prepared by Envirogen, Inc., 4100 Quakerbridge Road, Lawrenceville NJ 08648, for the Armstrong Laboratory Environics Directorate (AL/EQ), Suite 2, 139 Barnes Drive, Tyndall Air Force Base, Florida 32403-5323.

The major objective was to demonstrate the efficacy of a dual-stage bioreactor system for the treatment of groundwater contaminated with fuels and solvents. Under this SBIR Phase II, the number of chemicals to be treated during the field demonstration was expanded to include TCE, BTEX, and dichlorobenzenes (DCBs). A pilot-scale dual-stage bioreactor system was operated at Robins AFB GA to establish proof-of-concept and to develop operational and economic information for full-scale implementation. The system included a fluidized-bed reactor (FBR), an air stripper, and a TCE gas-phase bioreactor (GPR). Due to the enhanced performance of the FBR towards TCE, the demonstration essentially became a test of two independent pilot systems, one for treatment of contaminated water in the FBR and the second for treatment of contaminated air in the GPR. Operation of the system demonstrated effective treatment of not only BTEX and DCB, but also TCE. Over 210,000 gallons of contaminated groundwater were effectively treated during the demonstration. All hazardous chemicals were treated to concentrations near or below drinking water standards. An economic evaluation of the FBR to UV-peroxidation, air stripping with carbon adsorption, wet carbon adsorption and air stripping followed by PURUS adsorption suggests a significant cost savings over the life of a typical project.

The work was performed between 11 January 1993 and 30 December 1994. The AL/EQW project officer was Catherine M. Vogel. This is a Phase II Small Business Innovative research (SBIR) Report.

EXECUTIVE SUMMARY

Objective

The major objective of this project was to demonstrate the efficacy of a dual-stage bioreactor system for the treatment of groundwater contaminated with hydrocarbon-based fuels and solvents commonly found at Air Force installations. Initially, two key chemicals, benzene, and trichloroethylene (TCE), were identified as model targets for treatment. Benzene, a component of petroleum-based fuels, and TCE, a common solvent, represent two general classes of organic chemicals, nonchlorinated, and chlorinated, frequently found as groundwater contaminants. Under this Phase II SBIR award, the number of chemicals to be treated during the field demonstration was expanded to include TCE, benzene, toluene, ethylbenzene, xylenes, and dichlorobenzenes. This expanded list would further establish overall efficacy and performance for implementation at a wider range of contaminated sites. A pilot-scale dual-stage bioreactor system was operated at Robins AFB to establish proof-of-concept and to develop operational and economic information for full-scale implementation of this innovative technology.

Background

Through a combination of accidental discharges and previously accepted disposal practices, chemical contaminants have been introduced to soils and surface water at sites across the United States. At many sites, these chemicals now threaten groundwater supplies. A range of aggressive management strategies are being sought and implemented to minimize the waste being generated and to avoid adverse affects on the environment. Available physical/chemical treatment technologies for removal of organic chemicals from contaminated groundwater include activated carbon adsorption, air stripping, vapor extraction, and catalytic oxidation. These treatment methods can be costly and operationally complex and sometimes merely act to transfer the contaminants to another phase which requires subsequent treatment. Biological systems, including both *ex situ* and *in situ* approaches, offer an alternative cost-effective destruction technology for many classes of contaminants, including nonchlorinated and chlorinated organic chemicals.

Benzene, a constituent of jet aviation fuel is one contaminant commonly found at defense sites. Benzene is designated as a toxic pollutant under section 307 of the Clean Water Act and as a hazardous substance under section 311 of the Federal Water Pollution Control Act and further regulated by the Clean Water Act Amendments of 1977 and 1978. Benzene is highly mobile in the soil and may leach into groundwater. Federal drinking water surveys have determined that benzene is present at levels greater than 5.0 ppb in an estimated 1.3% of all groundwater systems and an estimated 3% of all surface water systems are contaminated at levels higher than 0.5 ppb. Although most public drinking water supplies are free of benzene, or contain <0.3 ppb, exposure can be very high from consumption of contaminated sources drawn from wells contaminated by leaky gasoline storage tanks, landfills, etc. Benzene and related aromatic hydrocarbons tend to be readily biodegradable under a range of environmental conditions.

Another prevalent organic chemical contaminant found in soil and groundwater is TCE. TCE contamination is aggravated by its relatively high solubility, high density, recalcitrance, and potential health risk. In addition, biotransformation of TCE in anaerobic subsurface regions can lead to the accumulation of vinyl chloride, a potent carcinogen. A 1989 compilation of the Final and Proposed NPL (National Priorities List) Sites listed 308 entries with TCE as a contaminant. The Department of Defense and Department of Energy have identified over 800 sites with TCE contamination. The Safe Drinking Water Act establishes allowable levels for TCE in drinking water at 5 µg/L (ppb). TCE represents a class of recalcitrant chemicals which often requires a unique set of conditions for biodegradation to occur.

Characterization of anaerobic and aerobic biotransformation of TCE and related volatile organic compounds (VOCs) has been ongoing for more than a decade. Under anoxic conditions, TCE undergoes reductive dehalogenation to dichloroethylene, vinyl chloride, and finally to ethylene, under optimal conditions. Under aerobic conditions, a broad variety of microorganisms co-metabolize TCE following growth with methane, propane, ammonia, phenol, or toluene. Though these bacteria apparently cannot grow with, or derive energy from the transformation of TCE, they can oxidize TCE to innocuous products, carbon dioxide, chloride ion, and other nonvolatile chemical species, using specific catabolic enzymes, either mono- or dioxygenases. A variety of bioreactor configurations have been studied to establish feasibility and to overcome limitations associated with co-metabolic degradation of TCE and related VOCs. ENVIROGEN has developed a gas-phase bioreactor (GPR) for the destruction of TCE, overcoming a variety of microbial and engineering limitations. Under typical operation, greater than 90% of the TCE is destroyed from a contaminated groundwater or airstream. The design of both laboratory-scale and pilot-scale systems balance the mass transfer of TCE from air to water with biodegradative capacity of the microorganisms.

This Phase II project was unique in that it specifically addressed the effective treatment of both nonchlorinated and chlorinated organic chemicals in a unified system. ENVIROGEN responded to a Small Business Innovative Research (SBIR) request for proposal, AF91-058, which specified development of a liquid phase treatment system to mineralize dilute concentrations of chlorinated and nonchlorinated organic chemicals in groundwater pumped from contaminated aquifers. A Phase I grant was awarded and successfully completed in January 1992. The Phase I work established the feasibility of biologically based treatment using model laboratory-scale bioreactor systems. Initial screening experiments demonstrated that: (1) TCE-degradative microorganisms were not capable of degrading benzene unless they had been induced following growth with toluene and/or phenol; (2) TCE did not inhibit benzene degradation over the range of concentrations tested and; (3) benzene-degradative organisms did not degrade TCE. These observations suggested that more than one microorganism would be required for the mineralization of both benzene and TCE in a dual-stage treatment system. Sequential degradation of benzene followed by TCE would be feasible since the TCE demonstrated no significant inhibitory effects on benzene degradation.

Scope

The overall scope of work for Phase II was to further develop and demonstrate a dual-phase treatment system at a contaminated site. A pilot-scale bioreactor system was used to treat groundwater contaminated with a wide range of nonchlorinated and chlorinated organic chemicals including, but not limited to, benzene and TCE. Ultimately, a full-scale commercial system will be designed for treating contaminated groundwater. The field trial was conducted at the base industrial area (OT20 site) at Robins Air Force Base. This site was selected jointly by the Air Force and ENVIROGEN. Two primary work phases followed site selection and refinement of the specific project problem definition: a) laboratory investigation, finalize pilot system design and preparation of pilot equipment and; b) field demonstration of the pilot-scale biotreatment system.

The first task was to develop a specific problem definition characterizing contamination at the selected demonstration site. Site characterization included identification of chemical contaminants, determination of contaminant concentrations, water analysis for mineral content, and other relevant chemical/physical characteristics. Systems and methods developed during Phase I were used to characterize the degradation of additional chemicals and to identify any adverse effects on overall performance. When required, microbial enrichments were performed to expand treatment capability thereby enhancing overall efficacy of this technology. Model laboratory-scale reactors were operated to establish operating parameters and to assess system performance. Final pilot-system design was determined, and equipment was prepared for operation at the demonstration site.

The main focus of this study was a field demonstration of the dual-stage bioreactor system. The pilot-scale system was operated by ENVIROGEN personnel using groundwater pumped from a contaminated aquifer. Concentrations of all targeted chemical contaminants were measured in both system feed and effluent streams. Several operating and performance parameters were monitored automatically, while others were manually tracked. Overall operation was evaluated for 8 weeks. Key parameters were evaluated for potential modifications to be implemented for the full-scale system design. Projected capital and operating costs were evaluated to determine overall economics.

Methodology

Standard methods for water analysis were used to monitor a variety of operating parameters including pH, biomass density, and nutrient concentrations. VOC concentrations were automatically monitored using gas chromatography (GC) systems. Two GC systems were employed, one for monitoring VOC concentrations in water and one for monitoring TCE concentrations in air. Both systems were routinely calibrated. Concentrations were determined by an external standard method.

Results

The base industrial area (OT20 site) at Robins Air Force Base was selected as the location for the Phase II demonstration. The test site was next to a fuel tank storage facility and the base industrial area. Groundwater has been monitored for several years and two separate contamination plumes have been mapped containing a wide range of chemical contaminants. Contaminated groundwater in the upper aquifer at the intersection of these two plumes contains greater than 100 µg/L of benzene, toluene, ethylbenzene, xylene (BTEX), dichlorobenzene (DCB), and TCE. The mixture of chemicals found at this site is representative of many contaminated sites.

Results from operation of a laboratory fluidized bed bioreactor (FBR) system demonstrated efficient removal of BTEX, DCB, and TCE. The granular activated carbon (GAC) in the FBR was saturated with chemicals before inoculation with degradative bacteria. Following inoculation and adaptation, effluent concentrations for all of the chemicals decreased. Once steady-state operation was achieved, greater than 90% of all chemicals and 80 to 90% of the TCE were degraded in the FBR. These results exceeded initial expectations and surpass results obtained in Phase I. Three major points were concluded from this test. First, chemical removal was biologically mediated and not a physical process. Second, there was an apparent adaptation of bacteria degrading toluene, ethylbenzene, and TCE. Third, there were no apparent toxic or inhibitory effects detected following long term operation. Several design and operating parameters were modified to enhance performance of the pilot FBR as a result of laboratory testing.

At the onset of this project, GPR operational stability was limited to less than 1 month, so laboratory efforts were concentrated on this issue. A change from batch to continuous operation was made resulting in enhanced operational stability. Performance was extended to beyond 10 months of continuous operation using laboratory systems. Several design and operating parameters were modified to enhance performance of the pilot GPR as a result of laboratory testing.

GAC adsorption isotherms were generated using a mixture of chemicals in concentrations expected during the demonstration. Carbon loading capacities were determined and used to predict chemical breakthrough at the concentrations and flow rates used during the demonstration. These results predicted that chemical breakthrough should occur in the absence of biological activity after two weeks of operation at 2 gpm using the weight of GAC loaded into the reactor. This information was used to plan the time schedule of the field demonstration.

The main focus of this project was to treat contaminated groundwater using a dual-stage bioreactor system. The field demonstration system included a fluidized-bed bioreactor (FBR), an air stripper, and a TCE gas-phase bioreactor (GPR). Due to the enhanced performance of the FBR towards TCE, the demonstration essentially became a test of two independent pilot systems, one for treatment of contaminated water in the FBR and the second for treatment of contaminated air in the GPR.

The FBR pilot was assembled and filled with GAC. Contaminated groundwater was pumped through the reactor for 4 weeks prior to addition of the bacterial inoculum. The system was operated at a flow rate of 2 gpm with an empty bed HRT of about 30 min, pH 6.7, 24°C, and 4.6 mg/L dissolved oxygen. Principal contaminants in the groundwater included benzene (46 µg/L), TCE (1,445 µg/L), toluene (40 µg/L), ethylbenzene (23 µg/L), xylene(s) (50 µg/L), and dichlorobenzene(s) (2,014 µg/L). The FBR effectively removed >97% of the 1,2-DCB and >95% of the BTEX from the water over the time period including preloading, steady state and spiked phases of operation. During this same time period, aqueous TCE concentrations were reduced by an average of 88% with a total mass balance demonstrating greater than 84% destruction beyond carbon adsorption in the FBR. Over 210,000 gallons of contaminated groundwater were treated in the pilot FBR during the field trial, with effluent quality close to drinking water standards. The FBR was capable of treating a wide variety of chlorinated and nonchlorinated aliphatic and aromatic chemicals typically found at both fuel storage and industrial areas. Pilot FBR performance was exceptional, with results fully consistent with the laboratory studies and exceeding those from the Phase I study. For this reason, the process flow sheet can likely be simplified from a dual-stage to a single-stage biological treatment system.

The GPR was essentially operated as an independent field demonstration for remediating TCE contaminated air. The GPR was capable of effectively treating TCE at concentrations up to 2,000 µg/L air. This system demonstrated enhanced stability approaching 2 months of operation before it was shut down. These results support the laboratory study which lasted 10 months before operation was terminated. Though actual field performance was lower than observed during laboratory testing, it is likely that removal efficiencies can be increased by increasing the biomass within the reactor. A full-scale GPR system has been designed to treat up to 500 cfm of contaminated air using an 11' diameter stirred tank reactor. This is a typical flow rate for soil vapor extraction systems.

Conclusions

Operation and performance of the FBR at Robins Air Force Base demonstrated effective treatment of not only BTEX and DCB, but also TCE. Full-scale FBR systems can be designed to treat up to 1,200 gpm of contaminated water, either groundwater or industrial wastewater, containing a wide range of chemical contaminants over a wide range of concentrations. A 5 ft diameter by 11 ft tall FBR design is capable of treating 100 gpm of groundwater contaminated with 15 ppm BTEX and 1 ppm TCE. Projected operating costs for these biological systems are less than 15% of the comparable costs associated with carbon adsorption systems. The FBR offers an economic alternative remediation option for treatment of contaminated waters.

Due to the high performance of the FBR, the vapor entering the GPR for treatment had to be spiked with TCE. TCE was reduced by an average of 75% in the GPR. This removal rate can be increased to over 90% by increasing biomass concentrations in the reactor. Removal efficiency was not optimized during the demonstration because the primary concern was long-term operational stability. Operational stability was successfully demonstrated with 10 months of continuous operation using the laboratory system, and 2 months using the field-pilot system. The economic analysis generated as part of these projects indicate typical savings in operating costs of 70 to 80% using the biological treatment system as compared to carbon adsorption.

In essence, two independent field demonstrations were successfully performed under this contract. Over 210,000 gallons of contaminated groundwater were effectively treated during the pilot demonstration. All hazardous chemicals were treated to concentrations near or below drinking water standards. An economic evaluation of this innovative FBR technology to UV-peroxidation, air stripping with carbon adsorption, wet carbon adsorption and air stripping followed by PURUS adsorption suggests a significant cost savings over the life of a typical project. Though the initial capital cost of the ENVIROGEN FBR system is higher than the most competitive option, air stripping with carbon adsorption, operating costs are significantly lower leading to a break even point at about 1.6 years. Biological treatment is a destructive technology, eliminating the hazard, whereas carbon adsorption would require additional treatment or containment of the contaminated, used carbon. If chemical concentrations are higher than those used in the cost estimates, operating costs for carbon adsorption would increase, whereas FBR and GPR operating costs will not change significantly. Biological treatment provides an economic, destructive technology for remediating contaminated air or water.

Recommendations

Selection of an appropriate remediation system depends on the specifics of the contaminated site and treatment requirements. As a result of this project, a number of bioreactor system options are available for treatment of contaminated groundwater and air. Specification of individual systems will depend on chemical concentration and composition, flow rates, and effluent treatment criteria at individual sites. The innovative technology demonstrated during this project is currently available for installation and operation for remediation of contaminated water, either surface or groundwater, and contaminated air originating from air stripping, air sparging, or soil vapor extraction operations.

TABLE OF CONTENTS

	<u>Page</u>
Section I:	
A. Introduction	1
B. Background.....	3
C. Scope/Approach	6
Section II:	
A. Wet Chemical Analysis.....	8
B. Organic Chemical Analysis.....	9
C. Bottle Assays.....	12
Section III:	
A. Laboratory Fluidized-Bed Reactor (FBR).....	13
B. Laboratory Gas-Phase Reactor (GPR).....	14
C. Pilot Demonstration Equipment.....	15
Section IV:	
A. Site Selection and Characterization.....	18
B. Biodegradation Assessment and Microbial Enrichments.....	20
C. Performance Evaluation of Laboratory FBR System.....	21
D. GAC Adsorption Isotherm Study.....	25
E. Performance Evaluation of Laboratory GPR System	29
F. Preliminary Pilot Demonstration Activities	31
G. Field Test Results from Operation of FBR System.....	33
H. Field Test Results from Operation of GPR System	44
I. Economic Evaluation for Full-Scale System	48
Section V:	
Conclusions	53
Section VI:	
Recommendations	54
Section VII:	
References.....	55

LIST OF TABLES

	<u>Page</u>
Table 1: Volatile and Semivolatile Organic Chemicals Monitored in FBR System	11
Table 2: Concentration and Composition of Contaminants in Groundwater at Robins AFB, Spring 1993	20
Table 3: Abiotic System Losses from the Laboratory FBR System	22
Table 4: Field Demonstration Time Line of Major Events	32
Table 5: FBR Pilot System Parameters Under Steady-State Operation (Day 0 through Day 55)	34
Table 6: Feed and Effluent Chemical Concentrations during Steady-State Operation of the Pilot FBR (Day 0 through Day 35)	35
Table 7: Feed and Effluent Chemical Concentrations during Spiked Steady-State Operation of the Pilot FBR (Day 36 through Day 58)	39
Table 8: Degradative Activity of Biomass Removed from Pilot FBR.....	42
Table 9: Methanol Extractable Organic Chemicals Removed from Pilot FBR	43
Table 10: Chemical Mass Balance for Key Contaminants during Operation of the Pilot FBR	44
Table 11: GPR Pilot System Parameters under Steady-State Operation (Day 0 through Day 60)	47
Table 12: Comparative Economics for FBR and Alternative Treatment Technologies.....	49

LIST OF FIGURES

	<u>Page</u>
Figure 1: Laboratory-Scale FBR System	14
Figure 2: Laboratory Gas-Phase TCE Reactor.....	15
Figure 3: Process Flow Diagram for Dual-Stage Biotreatment System	15
Figure 4: Map of OT20 Site at Robins Air Force Base, GA.....	19
Figure 5: Performance of Laboratory FBR for Benzene, Toluene, Ethylbenzene, and Xylenes Removal	23
Figure 6: Performance of Laboratory FBR for TCE, 1,2-Dichlorobenzene, 1,3-Dichlorobenzene, and 1,4-Dichlorobenzene Removal	24
Figure 7: Adsorption Isotherms for TCE, 1,2-DCB, and BTEX.....	26
Figure 8: Carbon Loading Estimates for Laboratory FBR System	28
Figure 9: Abiotic Loss Control from the Laboratory Gas-Phase Reactor ...	30
Figure 10: Stable Operation of the Gas-Phase TCE Reactor.....	31
Figure 11A: Performance of the Pilot FBR System	36
Figure 11B: Performance of the Pilot FBR System	37
Figure 11C: Performance of the Pilot FBR System	38
Figure 12: Abiotic Loss Control from the Pilot FBR System.....	39
Figure 13: Organic Loading to FBR GAC during Pilot Test.....	41
Figure 14: Abiotic Loss Control for Operation of the Pilot GPR.....	45
Figure 15: Steady-State Performance of the Pilot GPR.....	47
Figure 16: Process Flow Diagram for Standard FBR Systems.....	50
Figure 17: Life Cycle Costs of FBR and Carbon Adsorption Systems	50
Figure 18: Process Flow Diagram for Standard GPR.....	51
Figure 19: Life Cycle Costs of GPR and Carbon Adsorption Systems	52

LIST OF ABBREVIATIONS

cfm	cubic feet per minute
DCB	dichlorobenzene (three chemical isomers, 1,2-DCB, 1,3-DCB, and 1,4-DCB)
DO	dissolved oxygen
DOD	Department of Defense
ECD	electron capture detector
FBR	fluidized-bed bioreactor
FID	flame ionization detector
GAC	granular activated carbon
GC	gas chromatograph
gpm	gallons per minute
GPR	gas-phase bioreactor
HRT	hydraulic retention time
PID	photo ionization detector
ppb	parts per billion ($\mu\text{g/L}$)
ppm	parts per million (mg/L)
RAFB	Robins Air Force Base
SRT	solids retention time
TCE	trichloroethylene
VOC	volatile organic compounds

SECTION I: INTRODUCTION

A. INTRODUCTION

Through a combination of accidental discharges and previously accepted disposal practices, chemical contaminants have been introduced to soils and surface water across the United States. At many sites, these chemicals now threaten groundwater supplies. The Air Force is seeking aggressive management strategies to minimize the waste being generated and to avoid adverse affects on the environment. Over 800 sites have been identified as contaminated with chlorinated solvents. At these sites, TCE is the most frequently found, with JP-4 jet fuel commonly found as a co-contaminant. The Department of Defense (DOD), through the Installation Restoration Program (IRP), has identified a number of sites at military installations where the groundwater contains a variety of organic contaminants, including benzene and TCE, at concentrations exceeding federally allowed drinking water standards. Likewise, many contaminated industrial sites require remediation under Superfund and RCRA legislation. Aviation fuel, which contains benzene, is also a prevalent contaminant at many DOD installations worldwide. The Alternative Treatment Technology Information Center (ATTIC) database (Technical Resources, Inc.) alone lists at least 89 Records of Decision (RODs) that involve the cleanup of sites contaminated with TCE and 50 RODs for benzene alone. These sites are geographically distributed across the United States. Furthermore, the May 15, 1989 listing of the Final and Proposed NPL (National Priorities List) sites with TCE contains 308 entries. The cleanup of these sites and installations is a major challenge to and opportunity for, the development and implementation of cost-effective innovative technologies such as this dual stage bioreactor system.

TCE is a volatile chlorinated organic compound that has been used extensively as a solvent and degreasing agent. TCE contamination is aggravated by its relatively high solubility, high density, recalcitrance, and potential health risk. When released to soil, TCE near the surface can evaporate quickly. Any TCE that does not evaporate is highly mobile in the soil and can dissolve in groundwater or form DNAPLs (dense non-aqueous-phase liquids) which can pass through the aquifer and pool in the lower aquitard layer. In addition, biotransformation of TCE in anaerobic subsurface regions can lead to the accumulation of vinyl chloride, a

potent carcinogen (Reference 1). The Safe Drinking Water Act establishes allowable levels for TCE in drinking water at 5 µg/L (ppb).

Another contaminant commonly found at defense sites is benzene, a constituent of jet aviation fuel. Benzene is designated as a toxic pollutant under section 307 of the Clean Water Act, is designated as a hazardous substance under section 311 of the Federal Water Pollution Control Act and is further regulated by the Clean Water Act Amendments of 1977 and 1978. Benzene enters the atmosphere primarily from fugitive emissions and exhaust connected with its use in gasoline and from emissions associated with its production and use as an industrial intermediate. In addition, discharges into water can occur from industrial effluent sources and from accidental spills. When released to soil, benzene near the surface evaporates quickly. Any benzene that does not evaporate is highly mobile in the soil and may leach into groundwater. Federal drinking water surveys have determined that benzene is present at levels greater than 0.5 ppb in an estimated 1.3% of all groundwater systems and an estimated 3% of all surface water systems are contaminated at levels higher than 0.5 ppb (Reference 2). Although most public drinking water supplies are free of benzene, or contain <0.3 ppb, exposure can be very high from consumption of contaminated water drawn from wells contaminated by leaky gasoline storage tanks, landfills, etc.

Available physical/chemical treatment technologies for removal of organic chemicals from contaminated groundwater include activated carbon adsorption, air stripping, vapor extraction, and catalytic oxidation. These treatment methods can be costly and operationally complex and, can sometimes transfer the contaminants to another phase, which requires subsequent treatment. Biological systems, including both *ex situ* and *in situ* approaches, offer the possibility of a cost-effective destruction technology for many classes of contaminants, including nonchlorinated and chlorinated organic chemicals. The Department of Defense, through Tyndall AFB AL/EQ, has funded research and development for innovative, cost-effective technologies to remediate petroleum hydrocarbon and chlorinated solvent contamination.

B. BACKGROUND

Benzene and other hydrocarbon fuel components have been known to be aerobically biodegradable for many years. Biological treatment systems for fuel hydrocarbons have been demonstrated for both contaminated water and soils. However, these systems have not been capable of effectively treating TCE or other chlorinated organic chemicals that co-contaminate many sites. ENVIROGEN has developed a fluidized-bed bioreactor (FBR) designed to treat a wide range of contaminants at flow rates up to about 1,000 gpm and concentrations up to 1,000 mg/L total organics. The long solids retention time (SRT) attainable in the FBR minimizes the impact of toxic or inhibitory feed concentrations on system performance. Tolerance to such conditions is achieved by promotion of physical/chemical adsorption through the use of granular activated carbon (GAC) as the fluidization support material. The use of GAC in the FBR provides the additional benefits of rapid startup and enhanced performance, allowing removal of recalcitrant chemicals. Use of ENVIROGEN's proprietary bubbleless oxygenation system minimizes or eliminates losses of volatile organic chemicals to the atmosphere. The FBR system has a high biomass holding capacity resulting in high volumetric performance. An FBR was selected as the first-stage system for treatment of the more easily degradable hydrocarbon components, such as benzene, found at the selected site.

Characterization of the anaerobic and aerobic biotransformation of TCE and related volatile organic compounds (VOCs) has been ongoing for more than a decade (Reference 3). Under anoxic conditions, TCE acts as an electron acceptor and undergoes reductive dehalogenation by a nonspecific mechanism. Under aerobic conditions, a broad variety of microorganisms co-metabolize TCE following growth with methane, propane, ammonia, phenol, or toluene (References 4 to 14). Although these bacteria cannot grow with, or derive energy from TCE, they can oxidize TCE to innocuous products (carbon dioxide, chloride ion and other nonvolatile chemical species) using specific catabolic enzymes, either mono- or dioxygenases.

A variety of bioreactor configurations have been studied to overcome limitations associated with co-metabolic degradation of TCE and other chlorinated VOCs (References 15 to 22). These designs have attempted to minimize competitive interactions between the primary substrate and the co-metabolite, TCE, by temporal or spatial separation of growth with the primary substrate from treatment of the co-metabolite, TCE. Most of the early work focused on the use of pure microbial strains under aseptic conditions for short times of operation. The results of these studies helped define critical operating conditions and characterized limitations inherent with each system. Although laboratory-scale systems have demonstrated initial success in TCE degradation, there has been limited success using pilot-scale demonstration units.

Development of ENVIROGEN's gas-phase bioreactor for the destruction of TCE has been an iterative process. To date, ENVIROGEN has demonstrated both laboratory-scale and pilot-scale bioreactors for the destruction of TCE. This bioreactor configuration was designed to balance the mass transfer of TCE from air to water with biodegradative capacity. Under contract with the Department of Energy, laboratory reactors typically demonstrated greater than 90% destruction of TCE from a contaminated groundwater or air stream (Reference 23). This bioreactor has been demonstrated to perform equally well with several different aromatic degrading bacterial strains in the system. The first economic analysis generated as part of this study indicated typical savings of greater than 50% as compared to carbon adsorption (Reference 23). In related work, a mixture of 9 chlorinated organic compounds was treated using a laboratory-scale reactor. On average, 80% of the TCE, *t*-1,2 DCE, and 1,1 DCE was degraded in this mixture. As expected, PCE, chloroethanes, and chloromethanes were not degraded by the microorganisms used for the study. This developmental work focused on treatment of chlorinated hydrocarbons and not on mixtures of nonchlorinated and chlorinated organic chemicals defined in this project.

Effective treatment of both nonchlorinated and chlorinated organic chemicals in a unified system poses potential problems that this project was designed to identify and address. ENVIROGEN responded to a Small Business Innovative Research (SBIR) request for proposal, AF91-058, which specified development of a liquid phase treatment system to mineralize dilute concentrations of chlorinated and nonchlorinated organic chemicals in

groundwater pumped from contaminated aquifers. A Phase I grant was awarded and successfully completed in January 1992. This Phase I project established the feasibility of biologically based treatment using model laboratory-scale bioreactor systems. Initial screening experiments demonstrated that: (1) TCE-degradative microorganisms were not capable of degrading benzene unless they had been induced following growth with toluene and/or phenol; (2) TCE did not inhibit benzene degradation over the range of concentrations tested and; (3) benzene-degradative organisms did not degrade TCE (Reference 24). These observations suggested that more than one microorganism would be required for the mineralization of both benzene and TCE in a dual-stage treatment system. Sequential degradation of benzene followed by TCE would be feasible since the TCE demonstrated no significant inhibitory effects on benzene degradation.

Two laboratory-scale units were fabricated to model the dual-stage treatment system and each was tested for its ability to mineralize benzene and TCE (Reference 23). A first-stage fixed-film unit performed as designed, resulting in the destruction of benzene from an average of 45 ppm to below 1 ppm in the liquid stream. Typically, greater than 96 percent biodegradation of benzene from an artificially contaminated groundwater stream was observed over a 2-month period of continuous operation. The nondegraded benzene exited the reactor in either the air or liquid effluent streams. TCE in the contaminated groundwater elicited no significant inhibitory or toxic effects on benzene degradation. As expected with this first-stage unit, there was minimal activity against TCE. The liquid exiting the first-stage unit was stripped to transfer the TCE and "residual" benzene into the air phase. Benzene loads to the second-stage reactor were lowered in relationship to TCE loads to simulate the effluent from a moderately successful first-stage unit operation. The second-stage gas-phase bioreactor biodegraded >90 percent of the TCE and >90 percent of the residual benzene loads over the 6 days of testing. There were no apparent inhibitory effects of benzene on TCE degradative performance. Following first-stage treatment and subsequent stripping, the levels of both benzene and TCE were below the discharge limits of 5 ppb in the treated groundwater effluent. Results from this study demonstrated feasibility of the concept and provided operational and design parameters for a pilot-scale system.

C. SCOPE/APPROACH

The overall scope of work for this Phase II project was to develop and demonstrate ENVIROGEN's dual-phase biotreatment system using contaminated groundwater at an Air Force Base site. A pilot-scale bioreactor system would be used to treat groundwater contaminated with a wide range of nonchlorinated and chlorinated organic chemicals including, but not limited to, benzene and TCE. Ultimately, a full-scale commercial system would be designed for treating contaminated groundwater. The field trial was to be conducted at a site selected by the Air Force and agreed to by ENVIROGEN. Two primary work phases followed site selection and refinement of the specific project problem definition: a) laboratory investigation, finalize pilot system design and assembly of pilot equipment and; b) field demonstration of the pilot-scale biotreatment system.

The first task was to develop a specific problem definition by characterizing contamination at the selected demonstration site. Site characterization included identification of chemical contaminants, determination of contaminant concentrations, water analysis for mineral content and other relevant chemical/physical characteristics. It was assumed that additional hazardous chemicals, other than benzene and TCE, would be found and their treatment would be desired. Systems and methods developed during Phase I were used to characterize the degradation of key chemical contaminants found at the site and to identify any adverse effects on overall performance. If required, microbial enrichments would be performed to expand treatment capability, thereby enhancing overall efficacy of this technology. Model laboratory-scale reactors were operated using contaminated groundwater to establish operating parameters and to assess system performance. Final system design was determined and equipment was prepared for operation at the demonstration site.

The main focus of the proposed work was the treatment of contaminated groundwater at the selected site using a dual-stage bioreactor system. The pilot-scale system was operated by ENVIROGEN personnel using groundwater pumped from a contaminated aquifer. Concentrations of all targeted chemical contaminants were measured in both system feed and effluent streams. Several system operation and performance parameters were monitored automatically while others were manually tracked. Overall operation was evaluated for 8 weeks. Key design

parameters were evaluated for potential design modifications to be implemented for the full-scale system design. Projected capital costs and operating parameters were evaluated to determine the cost-effectiveness of this biotreatment technology.

The primary tasks associated with this proposal included: (1) selection of an appropriate test site; (2) laboratory testing to establish modifications to equipment design or operation resulting from additional chemicals, other than benzene and TCE, found at the site; (3) preparation, shipment, and assembly of equipment at the selected site; (4) operation of the pilot system; (5) demobilization of the pilot system and; (6) preparation of a final report. This document summarizes the results of these activities.

SECTION II: METHODOLOGY

A. WET CHEMICAL ANALYSIS

A variety of wet chemical analyses were used to track key operating parameters during all phases of work. Primarily, these methods were used to track the concentrations of biomass, nutrients, and chemicals critical to stable system operation.

Biomass concentrations were determined either by monitoring turbidity (absorbance at 550 nm) or by determining protein concentration (BCA, Pierce Chemical). Both methods quantified absorbance using a Spectronic 20 (Bausch and Lomb). Periodic correlations between turbidity and protein concentration were determined to assure accuracy of the turbidity method. Biomass concentrations in suspensions which were flocculant or nonhomogeneous were determined exclusively using the protein assay method.

Ammonia concentrations were determined in one of two ways. The first was a distillation and titration method. The sample was first adjusted to pH >9.5, then distilled. The distillate was collected in a boric acid indicator solution, then titrated with 0.02 N sulfuric acid solution. A calculation performed on the volume titrated against the volume of sample distilled yields the concentration of ammonia in the sample. The second method was performed in the field and utilized a colorimetric kit specific for ammonia (Aquarium Pharmaceuticals, Inc., Chalfont, PA). A 5 mL sample was added to a test tube containing 8 drops of a modified Nessler Reagent. The color generated was compared to a test chart. If required, samples were diluted to fall within the range of the assay, 0 to 7 ppm ammonia.

Phosphate concentrations were determined in one of two ways. The first method was performed at ENVIROGEN's customer service laboratory. Phosphate was quantified using ion chromatography (Dionex). A filtered aliquot of sample, 25 to 250 μ L, was pumped through an ion exchange column where the anions of interest were separated. The sample ions are selectively eluted off the separator column and onto a suppresser column. The eluent ions are neutralized and the sample ions are converted to their corresponding strong acids which are detected in a conductance cell. The ion chromatograph was calibrated with standard solutions containing known concentrations of the anion(s) of interest. The second method was performed in the field and used a colorimetric kit specific for

orthophosphate (Hach Co., Loveland CO). A test tube was rinsed with distilled water, then 0.5 mL of a filtered sample was added followed by 4.5 mL distilled water to give a total volume of 5.0 mL. One phosphomolybdate reagent powder pillow was added to the sample and the contents of the tube were mixed. The color generated was compared to a color wheel. If required, samples were diluted to fall within the range of the assay, 0 to 50 ppm PO_4^{3-} .

Phenol concentrations and rates of phenol degradation were determined using a modified colorimetric assay. A 1 mL sample was transferred to a plastic microfuge tube with 25 μL of 2% 4-aminoantipyrine and 50 μL of 2 N NH_4OH . The contents were mixed, 25 μL of 8% $\text{K}_3\text{Fe}(\text{CN})_6$ were added and the contents were again mixed. The tube was then centrifuged to pellet out suspended solids and the absorbance of the supernatant was determined at 500 nm using a spectrophotometer. The concentration range of the assay was between 5 and 100 μM (approximately 0.5 and 10 ppm). If the concentration was above this range, the sample was diluted.

B. ORGANIC CHEMICAL ANALYSIS: GAS CHROMATOGRAPHY

Initial characterization of site water was performed by ENVIROGEN's EPA certified analytical laboratory using standard methods. Major contaminants were identified using authentic standards and mass spectroscopic analysis.

ENVIROGEN used two SRI Gas Chromatographs (SRI GC) (SRI Instruments Inc., Torrance, CA) for automated analysis of organic chemicals entering and exiting the two reactor systems. Each GC was interfaced to an IBM-compatible personal computer and operated using PeakSimple III software (SRI Instruments Inc., Torrance, CA). Both GCs were equipped with 16-position stream selection valves (Valco Instruments Co. Inc., Houston, TX) which allowed for sampling and analysis from both feed and exit streams (though the system had the capacity of monitoring more locations). Sample transfer lines were 1/8-inch OD Teflon[®]. Samples were collected by drawing liquid or gas through the sample line into the sample loop on the GC. At least three sample line void volumes were passed through the sampling system to assure collection and analysis of a representative sample. Sample lines were periodically disconnected from their sample ports and monitored to assure that there was no sorption, desorption, or contamination issues with the automated system.

Performance of the FBR was monitored using an SRI model 9300 GC equipped with flame ionization (FID) and photoionization (PID) detectors in series and incorporated a purge and trap for analysis of volatile organic chemicals in water samples. The GC used a 105-meter stainless steel MXT-624 0.53 mm I.D. capillary column (Restek Corp., Bellefonte, PA). Each GC run took 90 minutes which included sampling, purge and trap sequence followed by injection and chromatographic separation of the chemicals. Nitrogen was used as the carrier gas with an initial column temperature of 45°C and a final temperature of 200°C. Primary standards were prepared for the 10 sets of compounds listed in Table 1. Two of the xylenes co-eluted, *m*- and *p*-xylene and were quantified together. Certified drinking water standard mixtures, DW-VOC Mix #1 and #2, were also purchased (Restek Corp., Bellefonte, PA) to confirm both retention times and quantitation. This also allowed for the identification of additional chemicals for which primary standards were not prepared, including vinyl chloride, trans-1,2-dichloroethylene, cis-1,2-dichloroethylene, 1,1-dichloroethylene, 1,2-dichloroethane, 1,1,2-trichloroethane, 1,2-dichloropropane, carbon tetrachloride, chlorobenzene, and styrene. The GC was equipped with a 5 mL sample loop which gave the detection limits listed in Table 1 using a peak area reject value of 100 units. Check standards were run at least twice each week. The FID demonstrated no shift in standard curve during the demonstration and this detector was used exclusively for quantifying chemical concentrations using an external standards method. If the sample concentration was greater than the highest standard, a split injection, both sample and standard, was used for quantification. This was necessary only during the spiking experiment. A water blank was run between feed and effluent samples to clean the purge and trap unit and to assure that there was no cross contamination by the higher concentration sample. The PID was used to confirm the identity of the aromatic and chloroethene components and assure that they were quantified correctly.

TABLE 1: VOLATILE AND SEMIVOLATILE ORGANIC CHEMICALS MONITORED IN FBR SYSTEM

chemical	abbreviation	retention time (min)	detection limit ($\mu\text{g/L}$)
benzene		41.9	2
trichloroethylene	TCE	47.6	10
toluene		57.3	2
tetrachloroethylene	PCE	60.8	12
ethylbenzene		66.7	3
<i>m,p</i> -xylene		67.3	1
<i>o</i> -xylene		68.1	2
1,3-dichlorobenzene	1,3-DCB	69.4	4
1,4-dichlorobenzene	1,4-DCB	69.8	3
1,2-dichlorobenzene	1,2-DCB	71.7	3

Performance of the GPR was monitored using an SRI model 8610 GC, equipped with electron capture (ECD) and FID detectors in series for analysis of volatile organic chemicals in air samples. A 30-meter VOCOLTM 0.53 mm ID Capillary Column (SUPELCO, Bellefonte, PA) was used for separation of the volatile organic compounds studied. Nitrogen was used as the carrier gas with an isothermal operation at 45°C and a rapid temperature ramp and bake at the end of each run. The inlet and outlet vapor streams were equipped with a liquid trap constructed of glass to eliminate liquid carryover to the GC. A 50 μL sample loop volume was used for analysis. Primary standards were prepared for TCE and the ECD was used exclusively for analysis since no other chemicals were added to the feed gas. Check standards were run at least twice a week. The ECD demonstrated no shift in standard curve during the demonstration and TCE was quantified using an external standards method. The detection limit for TCE was 1 $\mu\text{g/L}$ air. On several occasions, the moisture traps were overloaded due to foam in the reactor. On these occasions, the sample lines were cleaned and the data analyzed and rejected if contamination was suspected.

C. BOTTLE ASSAYS: ACTIVITY DETERMINATIONS AND GAC ISOTHERM

Degradation of targeted chemicals was determined using standardized assay methods developed at ENVIROGEN. Either a suspension of cells from a pure culture or a suspension of biosolids removed from one of the reactor systems was added to a 50 mL serum bottle. Biomass concentrations were determined by either monitoring turbidity (absorbance at 550 nm) or protein concentration (BCA Pierce Chemical). The chemical or mixture was then added to the bottle which was immediately sealed with a Teflon[®]-lined septum and shaken. Negative controls, consisting of either buffer alone or buffer with killed cells, were used to monitor abiotic losses. Sodium azide (0.1%) or adjustment to pH 10 were used to inhibit biological activity. At defined time intervals, either 10 μ L of air headspace or 0.25 to 2.0 mL of liquid was withdrawn for quantitation using a gas chromatograph. Concentrations were calculated in comparison to external standards. When specific rates were calculated, the decrease in concentration was determined as a function of time and biomass concentration. This closed assay method allowed for accurate assessment of the extent of biodegradation and complete chemical mass balance under a wide variety of conditions in a short period of time.

GAC isotherm experiments were performed by adding 20 mL of buffer and 10 grams dry weight granular activated carbon (GAC) to a 50 mL serum bottle. Between 10 and 1000 μ L of a mixture of organic chemicals was then added to the bottle which was then sealed with a Teflon[®]-lined septum and shaken at 200 rpm for 24 hours. The mixture of organic chemicals was prepared in a mass ratio similar to that expected during the field demonstration. The GAC used came from the same batch as that used during the pilot demonstration in the FBR system. Following equilibration, 0.5 to 5.0 mL of water was removed and analyzed using a GC/FID equipped with a purge and trap.

SECTION III: TEST DESCRIPTION

A. LABORATORY FLUIDIZED-BED REACTOR (FBR)

Two laboratory-scale FBR systems were constructed of glass with Teflon[®] tubing and polypropylene fittings to minimize abiotic chemical losses. Each system had a total liquid volume of 4 liters and an empty bed volume of 800 mL (Figure 1). One reactor was used to monitor abiotic losses of chemicals from the system. GAC was added to give a settled bed volume of about 800 mL which was fluidized to 125% using a gear pump on the recycle line. Contaminated water was fed at 15 to 20 mL/min which resulted in an empty bed hydraulic retention time (HRT) of approximately 40 to 50 minutes. The empty bed HRT was calculated based on bed volume, feed flow rate and assumes no significant biodegradative activity is occurring in other wetted areas of the reactor. Dissolved oxygen levels were maintained above 2 ppm through a control system. Liquid level in the oxygenator was monitored using a reservoir monitor which opened a solenoid valve thereby maintaining a fixed volume of pure oxygen in the oxygenator. Liquid pH was automatically controlled between 6.8 and 7.0 through the addition of 5 N sodium hydroxide. Temperature was maintained between 22 and 30°C. Liquid samples for organic analysis were taken just prior to entry into the recycle line (feed) and from the upper liquid reservoir (effluent). Samples were collected either manually or automatically with analysis performed using a purge and trap equipped GC/FID as described above.

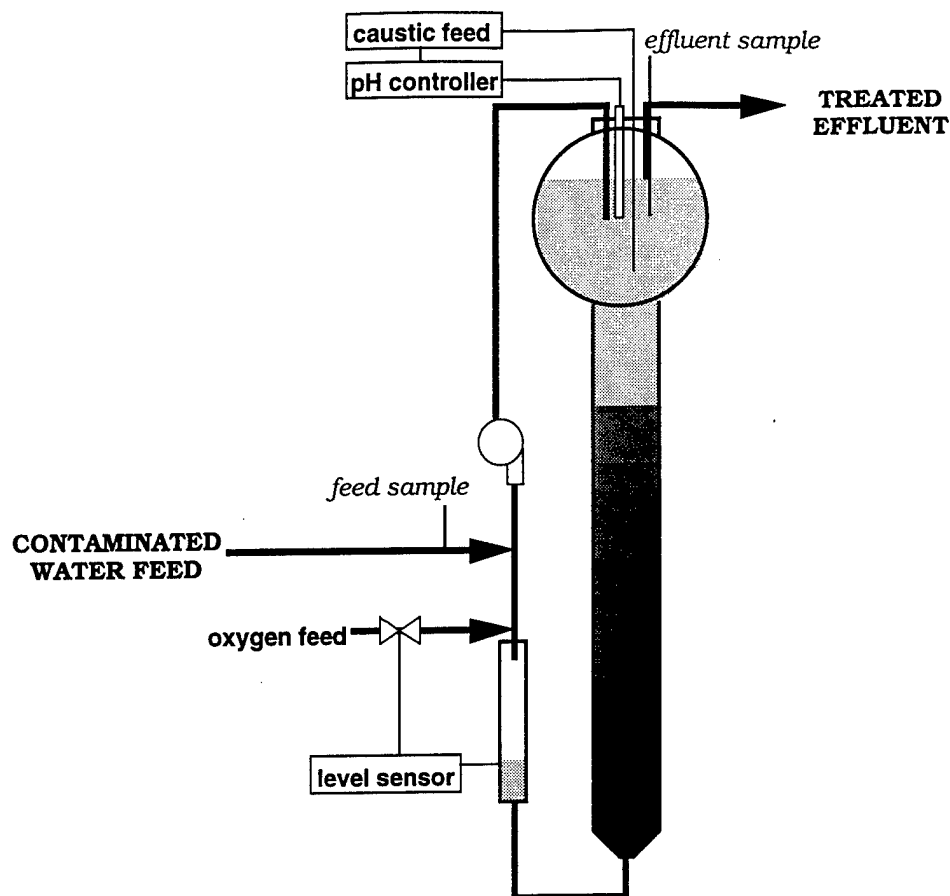


Figure 1: Laboratory-Scale FBR System

B. LABORATORY GAS-PHASE REACTOR (GPR)

The GPR system, with a working liquid volume of 2 liters, was constructed from glass, Teflon[®] and stainless steel to minimize abiotic losses of volatile organic chemicals (Figure 2). A proprietary nutrients mixture, containing nitrogen and phosphate, was added at a rate of about 0.7 mL/min which gave a hydraulic retention time of about 10 days. Liquid pH was automatically controlled between 6.8 and 7.0 through the addition of 5 N sodium hydroxide. Temperature was maintained at 28°C. Dissolved oxygen levels were maintained above 2 ppm without need for an automated control system. Air contaminated with TCE entered through a 1/8-inch tube at the bottom of the vessel and exited after passing through the liquid column with suspended bacteria. An automated gas sampling system was connected to both inlet and outlet gas streams and TCE concentrations were monitored by an automated GC/ECD as described above. Liquid samples were analyzed for TCE periodically using either extraction or purge and trap methods.

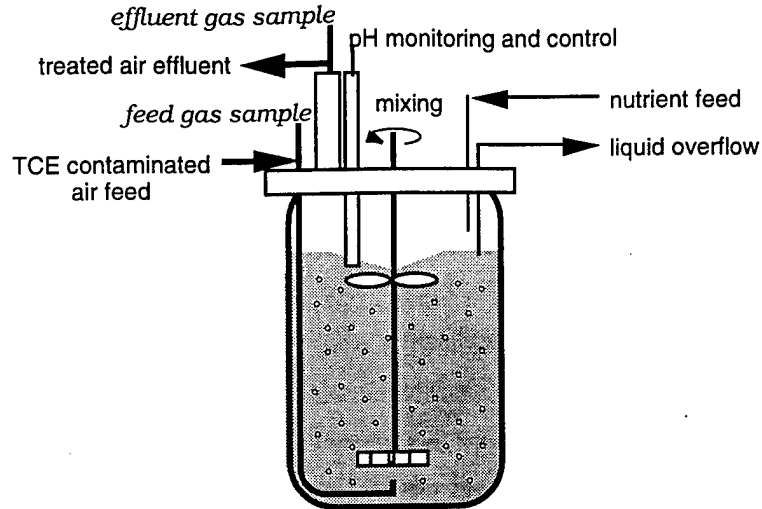


Figure 2: Laboratory Gas-Phase TCE Reactor.

C. PILOT DEMONSTRATION EQUIPMENT

The process flow diagram for the field pilot demonstration includes a first-stage fluidized-bed bioreactor (FBR), an air stripper, and a TCE gas-phase bioreactor (GPR) (Figure 3).

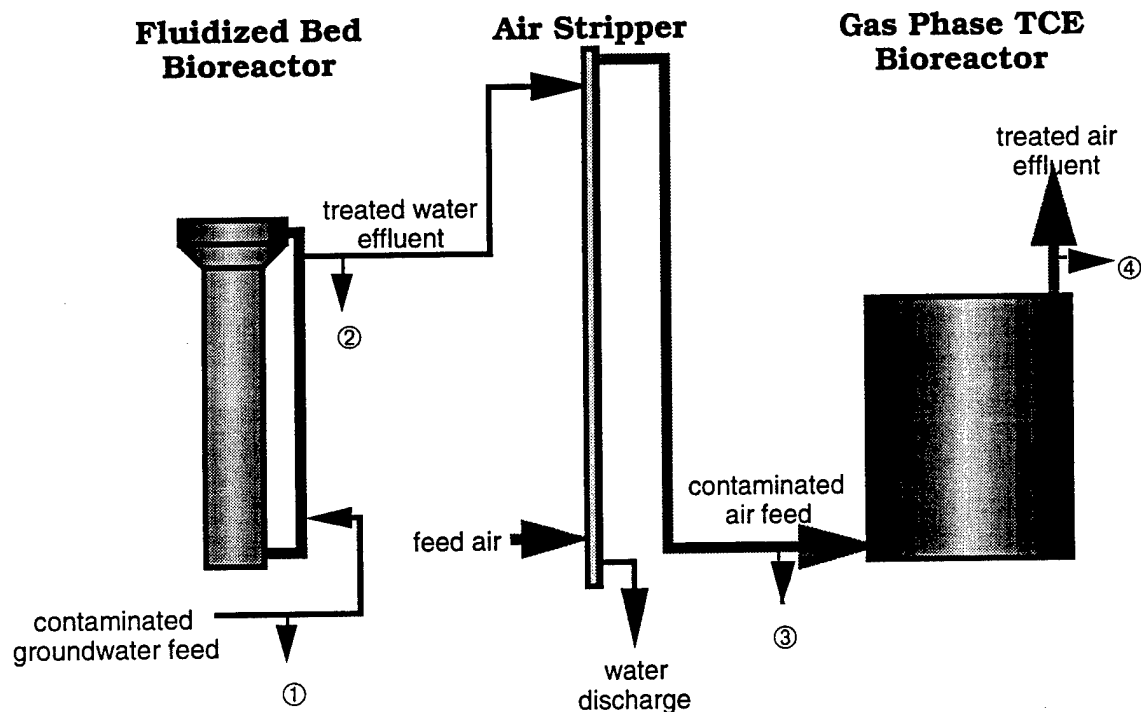


Figure 3: Process Flow Diagram for Dual-Stage Biotreatment System. Automated sampling and analysis locations are labelled; ① - FBR liquid feed, ② - FBR liquid effluent, ③ - GPR gas feed and ④ - GPR gas effluent.

The process flow diagram (Figure 3) shows the primary water and air flows for the two stage treatment system. Water was pumped from 2-inch diameter monitoring wells and combined in an expansion tank where the water was mixed and flow rates were controlled. The combined water flow then passed into the FBR. The treated effluent water was then transferred to the air stripper with the residual VOCs transferred to the vapor phase in the stripper. The contaminated vapor exiting the stripper was then passed into the GPR. The treated vapor exiting the GPR was vented to the atmosphere. Water required for operation of the GPR was drawn from the stripper. Water exiting both the stripper and the GPR was collected and discharged to the sewer which flowed into the base's industrial treatment plant.

ENVIROGEN's field pilot Fluidized-Bed Reactor system has approximate dimensions of 12.5-feet long by 5.5-feet wide by 15-feet high and weighs about 5,000 pounds. The reactor vessel is approximately 1 foot in diameter by 14-feet high with an empty bed volume of about 66 gallons. The equipment requires single-phase, 240-volt power rated at 30 amps. The vessel and piping are constructed of stainless steel to minimize fugitive chemical emissions and provide chemical compatibility with a wide range of chemicals. Both the feed water separator tank, located on the feed piping, and the solids recovery tank, located after the reactor vessel in the recycle line, can be used or bypassed as desired. All process controls and equipment are weatherproof, allowing for outside operation. The system was controlled and operation was monitored through a computer control and data logging system. Process control was accessed remotely via a dedicated phone line through the data acquisition system. Oxygen was supplied by compressed gas cylinders and DO levels were monitored and controlled either manually or automatically. System pH was monitored and controlled through the addition of either caustic or acid as required. Two of the three chemical feed tanks and delivery systems were used for addition of caustic, acid, and nutrients. Caustic was added as required and nutrient addition was set to a predetermined rate. Approximately 210 pounds of GAC, ENVG830, was used as the bed support. Bed height level was monitored and logged. Automatic bed height and temperature controls were available but not used. Any off-gas generated from the process was passed through an on-skid carbon trap. However, minimal off-gas was generated through the use of a proprietary bubbleless oxygenation system. The system is capable of handling up to 10 gpm liquid flow, depending on the specific

contaminants and concentrations present. Sample ports are located at key points through the system including feed, influent, effluent, and along the height of the reactor vessel. During this demonstration, the automated purge and trap GC system described previously was used to automatically monitor feed and effluent water streams.

ENVIROGEN's field pilot Gas-Phase Reactor system has approximate dimensions of 8-feet long by 8-feet wide by 11-feet high and weighs about 6,000 pounds. The reactor vessel is approximately 6 feet in diameter and 10-feet tall and holds approximately 750 gallons of liquid. The system was operated in a stirred-tank mode. The equipment requires three-phase, 480-volt power rated at 70 amps. The vessel and piping are constructed of either carbon or stainless steel to minimize fugitive chemical emissions and provide chemical compatibility with a wide range of chemicals. System pH was automatically controlled using caustic. The system has two on-skid and two off-skid chemical feed systems for addition of caustic, acid and/or nutrients. Reactor water temperature was controlled through an on-skid heater and refrigeration system. If required, all off-gas generated from the process can be passed through an on-skid carbon trap. The system is capable of handling up to 30 cfm air flow, depending on the specific contaminants and concentrations present. Sample ports are located at key points throughout the system, including gas influent, gas effluent, and liquid from the reactor vessel. During this demonstration, the automated gas analyzing GC system described previously was used to automatically monitor feed and effluent air streams.

SECTION IV: TEST RESULTS

A. SITE SELECTION AND CHARACTERIZATION

Robins Air Force Base (RAFB), located south of Macon, Georgia, was selected for this field demonstration site. RAFB is a logistics base, instrumental in the maintenance and repair of a variety of aircraft. The base industrial area (OT20 site), next to a fuel storage tank farm and the flight line, contains machine shops, electroplating facilities, and painting facilities (Figure 4). Three geological formations underlie the base including the Providence aquifer, the Cusseta unit and the Blufftown aquifer in descending order. The superficial aquifer material contains medium to coarse grained sands with interlayered thin clay lenses. The groundwater in the upper Providence aquifer is 13 to 27 feet below ground level and flows in an easterly direction. Two separate contaminant plumes have been characterized containing both chlorinated and nonchlorinated organic chemicals. One plume appears to originate from the industrial facilities and a second originates from the region of the fuel storage facility. A history of chemical use and disposal practices has implicated four buildings (140, 141, 142, and 181) as potential sources for the chemicals found in this area. Groundwater samples collected in March 1993 confirmed the presence of chlorinated and nonchlorinated organic chemicals in the upper aquifer (Table 2). The base industrial area (OT20 site) at Robins Air Force Base was selected as the location for the Phase II demonstration.

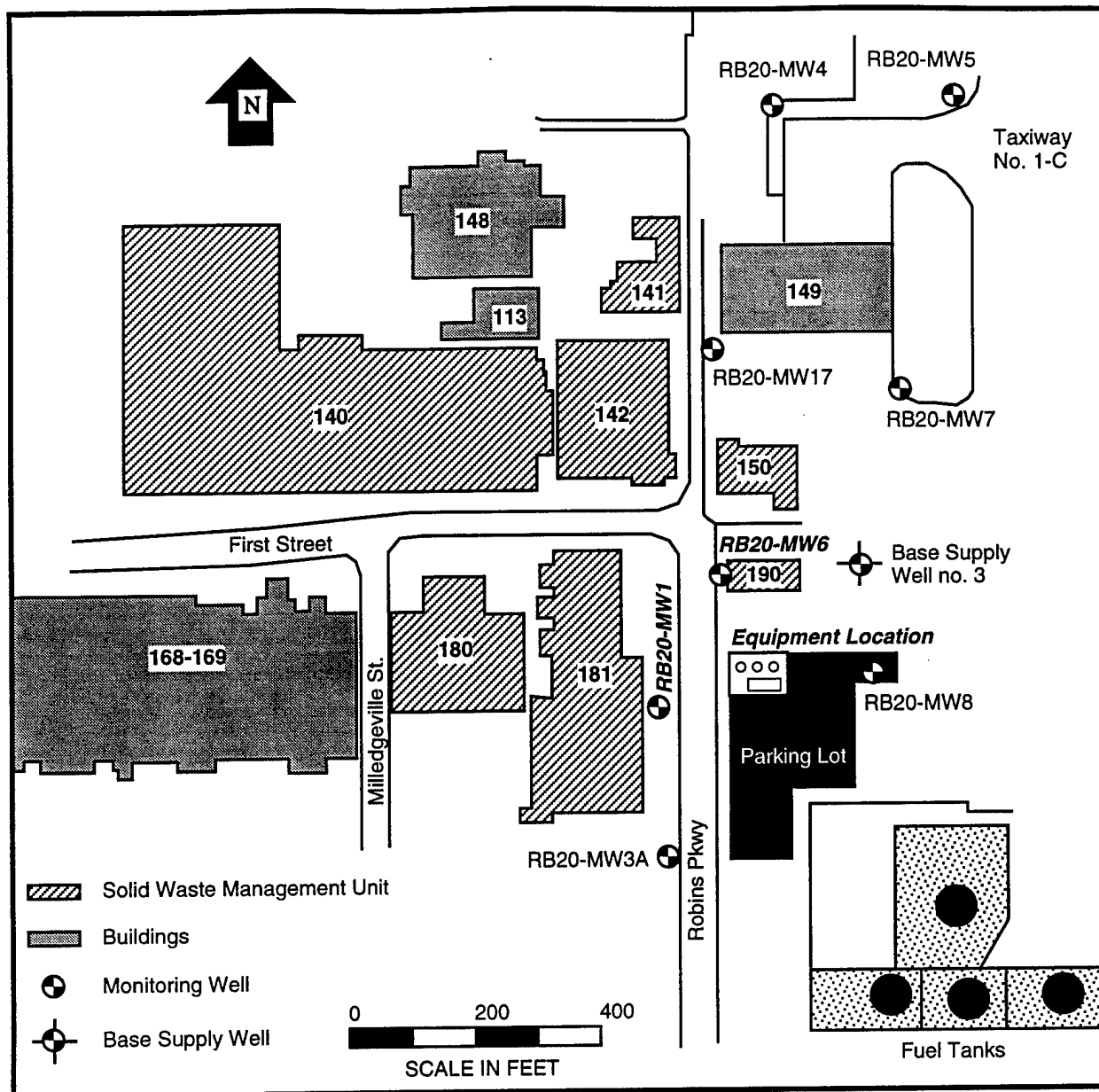


Figure 4: Map of OT20 Site at Robins Air Force Base, GA.

TABLE 2: CONCENTRATION AND COMPOSITION OF CONTAMINANTS IN GROUNDWATER AT ROBINS AFB, SPRING 1993

compound	MW-1 (ppb)	MW-6 (ppb)	MW-8 (ppb)
1,1-DCA ¹	74	10	
1,1,1-TCA ¹	120	3	
1,2-DCE	55	160	
TCE ²	2500	1500	510
PCE ¹	180	80	
benzene ²	14	270	520
toluene ²		810	2200
ethylbenzene ²		170	980
total xylene ²		1300	7000
1,3-dichlorobenzene ²		140	
1,4-dichlorobenzene ²		310	
1,2-dichlorobenzene ²		3300	160
naphthalene		110	600

1 - chemicals are not readily biodegradable aerobically

2 - chemicals used in laboratory testing

no entry indicates concentration less than 1.0 ppb

B. BIODEGRADATION ASSESSMENT AND MICROBIAL ENRICHMENTS

Several organic chemicals were identified at the OT20 site, in addition to those specified in the original request for proposal. Those chemicals with concentrations greater than 0.5 ppm included TCE, benzene, toluene, ethylbenzene, xylene(s), and dichlorobenzenes (DCBs). Laboratory testing was conducted to assess their impact on the design and operation of the dual-stage system. The initial testing program focused on microbial enrichments and laboratory-scale bioreactor studies. ENVIROGEN already possessed degradative bacteria capable of growth with most of the chemicals of interest, so work was focused on microbial enrichments for growth with DCBs. In addition, several degradative strains were obtained from Dr. Jim Spain of Tyndall AFB for evaluation. Water from RB20-MW6 was used to successfully enrich for bacteria capable of growth with all three DCB isomers as sole carbon and energy sources. Bottle assays

were employed to evaluate acute toxic or inhibitory effects of the various chemicals on degradation. No significant interactions were identified and all chemicals were degraded using contaminated groundwater collected from the site. Two laboratory-scale bioreactors were then used to evaluate long term performance characteristics for finalizing design and operation of the field system.

C. PERFORMANCE EVALUATION OF LABORATORY FBR SYSTEM

A fluidized-bed bioreactor (FBR) was selected for the first-stage reactor design as a replacement for the original static fixed-film design used during Phase I work. The granular activated carbon (GAC) based FBR possesses several outstanding operational features including rapid startup, high biomass holding capacity, automatic control of biofilm thickness, long solids retention, and uniform performance with variable feeds. Potential abiotic losses of chemicals from the FBR were experimentally determined. A water-filled reactor was operated in a flow-through mode without GAC. Sodium azide was added to about 0.1% to inhibit biological activity and a mixture of chemicals was added to feed water to mimic those expected at the test site. Samples were collected in duplicate from feed and effluent water over 7 days of operation. Though some losses were observed, the average recovery was about 90% for the set of chemicals tested (Table 3). Similar results were obtained for systems operated with GAC once steady-state chemical breakthrough had occurred, though it was more difficult to completely eliminate biological activity in those experiments.

TABLE 3: ABIOTIC SYSTEM LOSSES FROM THE LABORATORY FBR SYSTEM.

compound	[feed] (ppb)	[effluent] (ppb)	% recovery
benzene	440 ± 130	397 ± 64	90
TCE	5681 ± 1628	5036 ± 281	89
toluene	594 ± 194	556 ± 77	94
PCE	2445 ± 762	2198 ± 200	90
ethylbenzene	150 ± 53	137 ± 20	91
<i>m,p</i> -xylene	44 ± 21	44 ± 16	100
<i>o</i> -xylene	46 ± 16	51 ± 10	112
1,3-dichlorobenzene	389 ± 200	352 ± 48	90
1,4-dichlorobenzene	531 ± 254	480 ± 64	90
1,2-dichlorobenzene	3689 ± 1925	3043 ± 583	82

GAC free FBR system operated in flow-through mode with 0.1% sodium azide. Data was tabulated from 15 sets of samples collected over three days of operation.

A laboratory FBR was operated to establish performance characteristics. Before inoculation, the GAC bed support was saturated with the mixture of chemicals exceeding the sorption capacity of the carbon. The reactor was operated in 100 % recycle for 6 days prior to inoculation. Chemicals in the recycle liquid (equivalent to effluent samples) demonstrated steady-state breakthrough at concentrations close to feed concentrations (Figures 5 and 6). Degradative microbial populations were grown separately in shake flasks with either benzene, toluene, ethylbenzene, xylenes, or dichlorobenzenes as sole carbon sources. At time zero, a mixed inoculum was prepared from the separate cultures, added to the reactor, and allowed to attach to the GAC for 12 hours. Flow-through operation was then initiated using water amended with a mixture of chemicals to mimic conditions at the site. Contaminated water was fed to the reactor to give an empty bed hydraulic retention time of about 60 minutes. Following inoculation and flow-through operation, the concentrations of benzene, xylenes, and dichlorobenzenes all decreased in the treated effluent. For toluene, ethylbenzene, and TCE, effluent concentrations followed feed concentrations for one to three weeks. Following this apparent lag in degradative activity, effluent concentrations of toluene, ethylbenzene, and TCE declined significantly. For the duration of operation, effluent quality remained high with >90% degradation of all chemicals and 80 to 90% degradation of TCE.

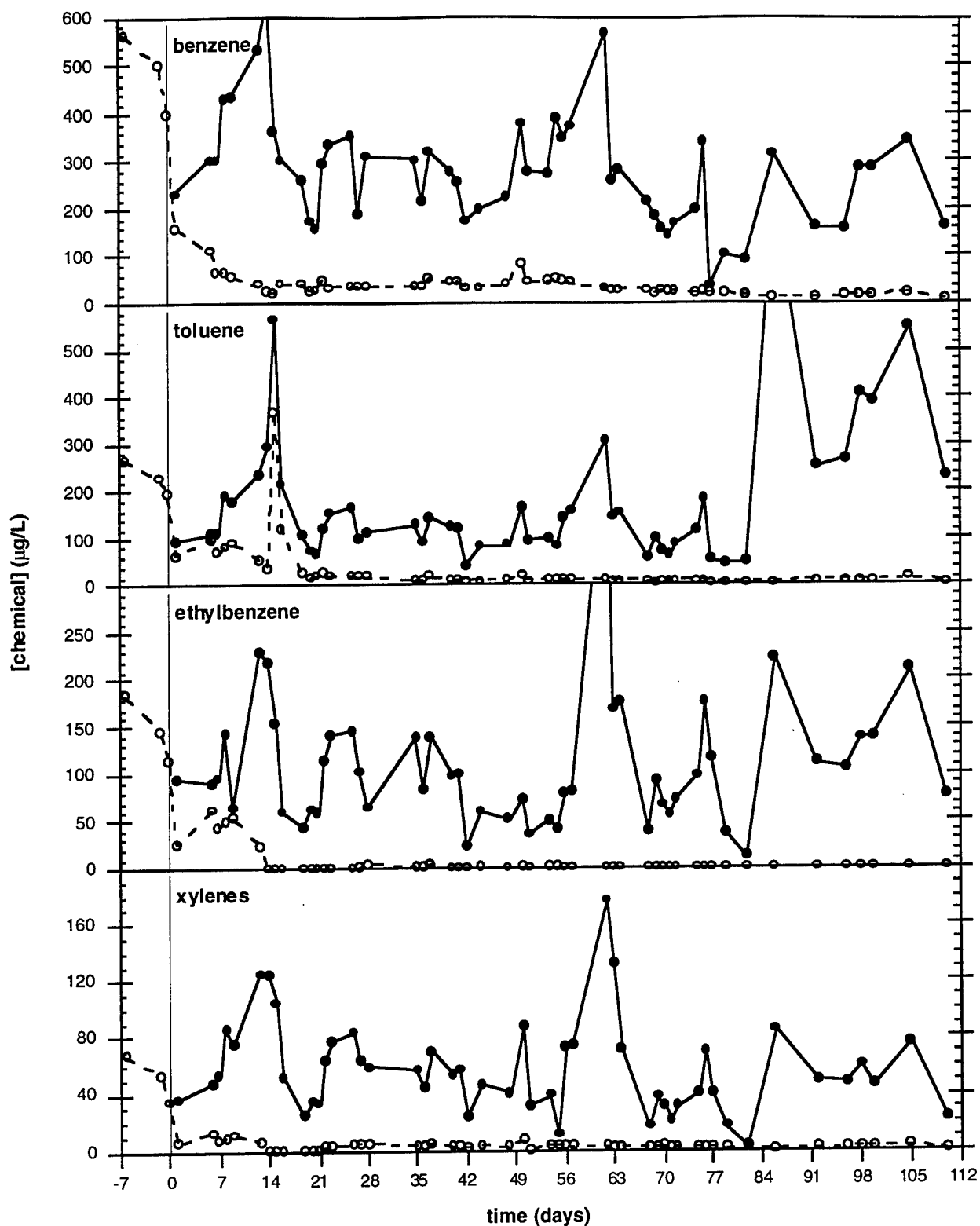


Figure 5: Performance of Laboratory FBR for Benzene, Toluene, Ethylbenzene, and Xylenes Removal. Chemical concentration was plotted against time of operation for contaminated feed (●) and treated effluent (○).

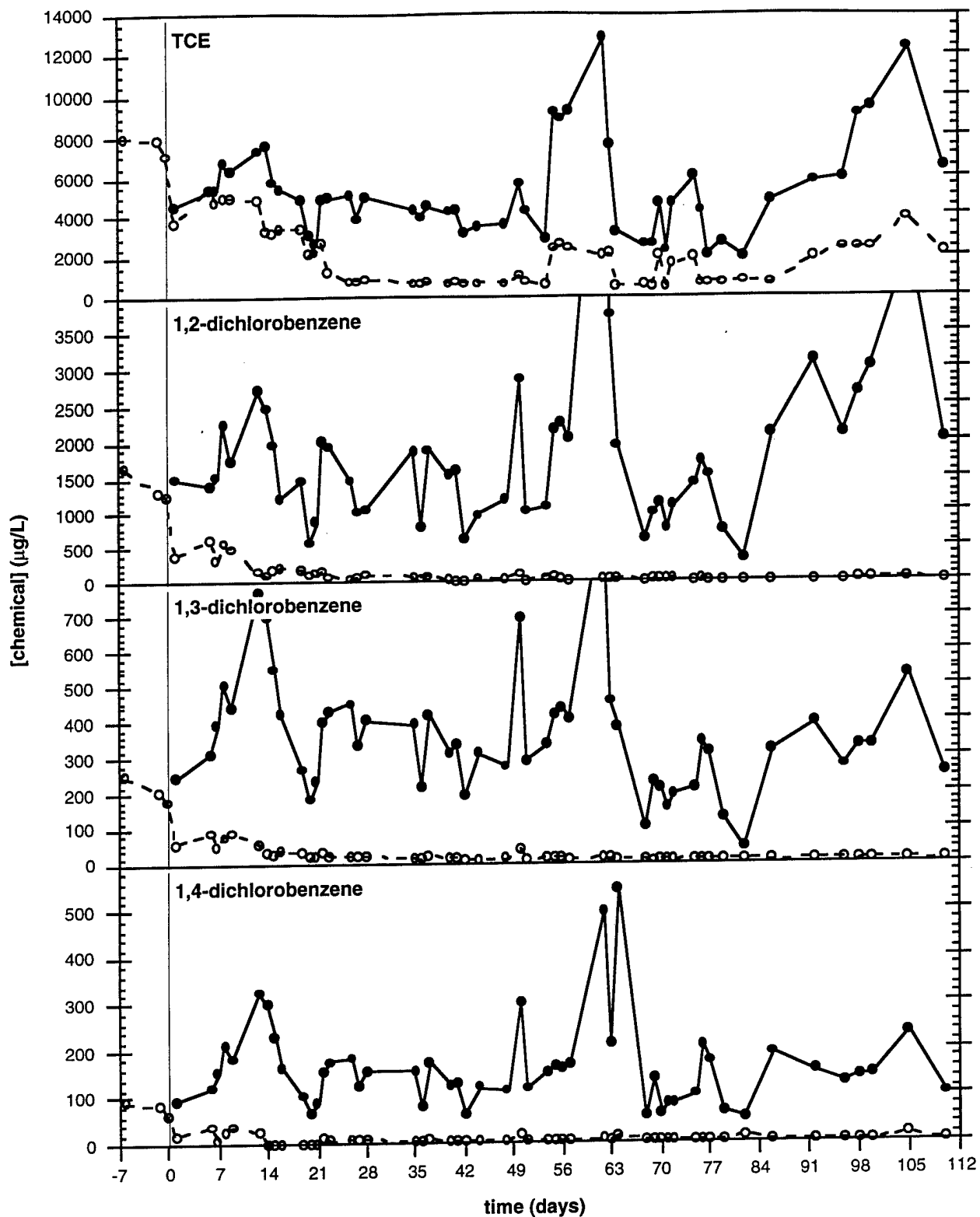


Figure 6: Performance of Laboratory FBR for TCE, 1,2-Dichlorobenzene, 1,3-Dichlorobenzene, and 1,4-Dichlorobenzene Removal. Chemical concentration was plotted against time of operation for contaminated feed (●) and treated effluent (○).

D. GAC ADSORPTION ISOTHERM STUDY

One central issue when using GAC as a bed support in a bioreactor concerns differentiation between chemical adsorption and biodegradation. Adsorption isotherms were determined using a mixture of chemicals representative of those at the test site and the GAC used in the pilot FBR. A second adsorption isotherm was generated using TCE as the sole contaminant. Isotherms were plotted for TCE, 1,2-DCB, and combined BTEX (Figure 7). Samples of GAC from the isotherm experiment using TCE as the sole contaminant were extracted with methanol to directly determine the amount of bound TCE and the extraction efficiency of this method. GAC samples were analyzed from three samples at low, medium and high TCE loadings with an average of 99% of the bound TCE recovered. Methanol essentially replaces the TCE bound to the GAC through an apparent competitive mechanism thereby releasing the TCE from the GAC. The isotherms were then used to predict chemical breakthrough in the absence of biological activity based on organic loading rates.

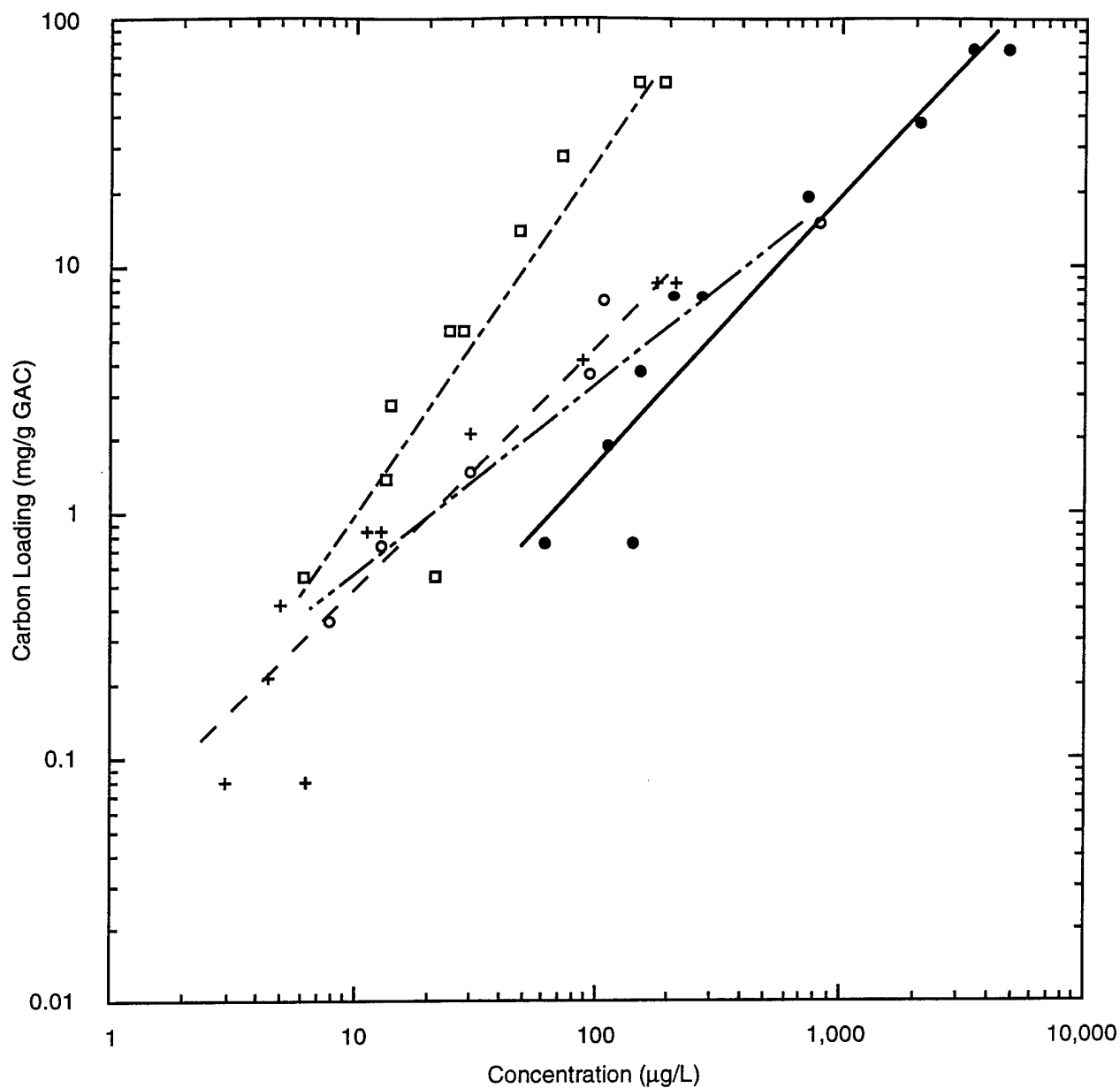


Figure 7: Adsorption Isotherms for TCE, 1,2-DCB, and BTEX. The carbon loading was plotted against the free aqueous concentration for TCE (●), 1,2-DCB (□), and BTEX (+) for individual components in a mixture comparable to that expected during the field demonstration and for TCE (○) as a single contaminant.

Organic loading on the carbon support was calculated and plotted for operation of the laboratory FBR (Figure 8). Since the carbon had been presaturated with the key chemicals found in the site water, the initial loading was assumed to be on the isotherm (circled cross). As contaminated water was fed to the reactor the loading increased with time. Loading rates were the product of the feed flow rate and the difference between feed and effluent concentrations. The plot clearly shows that as the bacteria grew and adapted to the chemical contaminants, the effluent concentrations dropped resulting in a carbon loading well above the isotherm. This trend was strongest for BTEX and DCB though also apparent for TCE. This presentation of data supports the conclusion that all major chemicals were being biodegraded and not just adsorbed to the GAC support matrix in the FBR system.

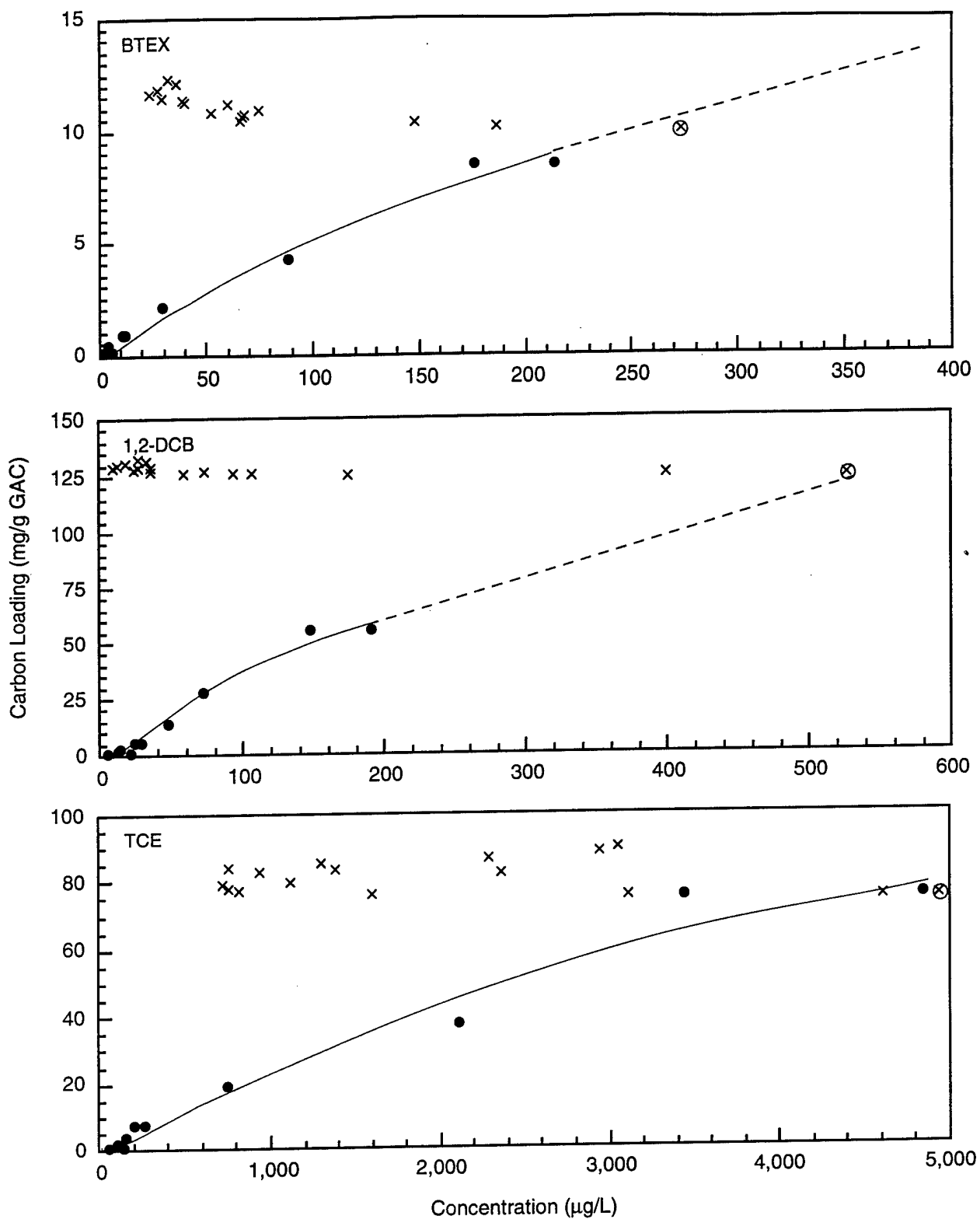


Figure 8: Carbon loading estimates for laboratory FBR system. The carbon loading was plotted against the free aqueous concentration for BTEX, 1,2-DCB, and TCE. The loading at time zero is indicated by the circled cross.

E. PERFORMANCE EVALUATION OF LABORATORY GPR SYSTEM

Extensive testing and development of the gas-phase TCE bioreactor had been completed prior to award of this Phase II project. Several microbial and engineering issues had been successfully overcome, leading to the demonstration of a pilot reactor at an aerospace site in the northeast. This gas-phase bioreactor used a strain of bacteria which grew with either toluene or phenol and co-metabolically degraded TCE. Typically, 90% of the TCE was degraded from a contaminated gas stream entering the reactor at 4 to 20 cfm with 150 to 250 μg TCE/L air. However, a major operational issue remaining was long-term operational stability of the bacterial population. This issue was addressed specifically during the laboratory phase of this contract.

A bacteria-free control was performed to determine TCE recovery and abiotic losses from the system. TCE-contaminated air was bubbled through the reactor under standard conditions until steady-state concentrations in the water and air phases were attained. The reactor was run in this mode for 3 days to determine both variability in the feed system and abiotic system losses (Figure 9). Essentially all of the TCE entering the reactor could be recovered in the effluent gas stream in the absence of biodegradative activity.

The reactor vessel was then sterilized in an autoclave and inoculated with a pure culture of *Pseudomonas cepacia* G4 grown with phenol as the sole carbon source. The reactor was operated at a 5-day hydraulic retention time using a feed of phenol in nutrient solution at pH 7.0 and 28°C. Once significant biomass levels were attained, TCE contaminated air was introduced to the reactor. Typically, between 200 and 600 μg TCE/L air entered the reactor at an air flow rate of 70 mL/min with effluent concentrations close to, or below detection limits (Figure 10). This was equivalent to a 4 cfm flow rate with the pilot reactor system. Overall TCE removal efficiencies exceeded 95% though there were several minor operational upsets which included plugged feed lines and interruptions in electrical service. Following each event, biological activity in the reactor recovered without amendments to, or replacement of, the bacteria. The bacterial composition of the reactor became a mixed culture after the first week of operation. Bacterial plate counts demonstrated several colony morphologies though specific rates of phenol degradation remained within 30 to 50% of maximal rates obtainable with pure cultures. These reactors continued to operate for over

10 months under similar operating conditions. Clearly, modifications made in gas-phase bioreactor operation led to stable and reliable performance for extended time periods. This was a resounding success with an increase in operating life from 4 weeks to over 10 months of continuous operation.

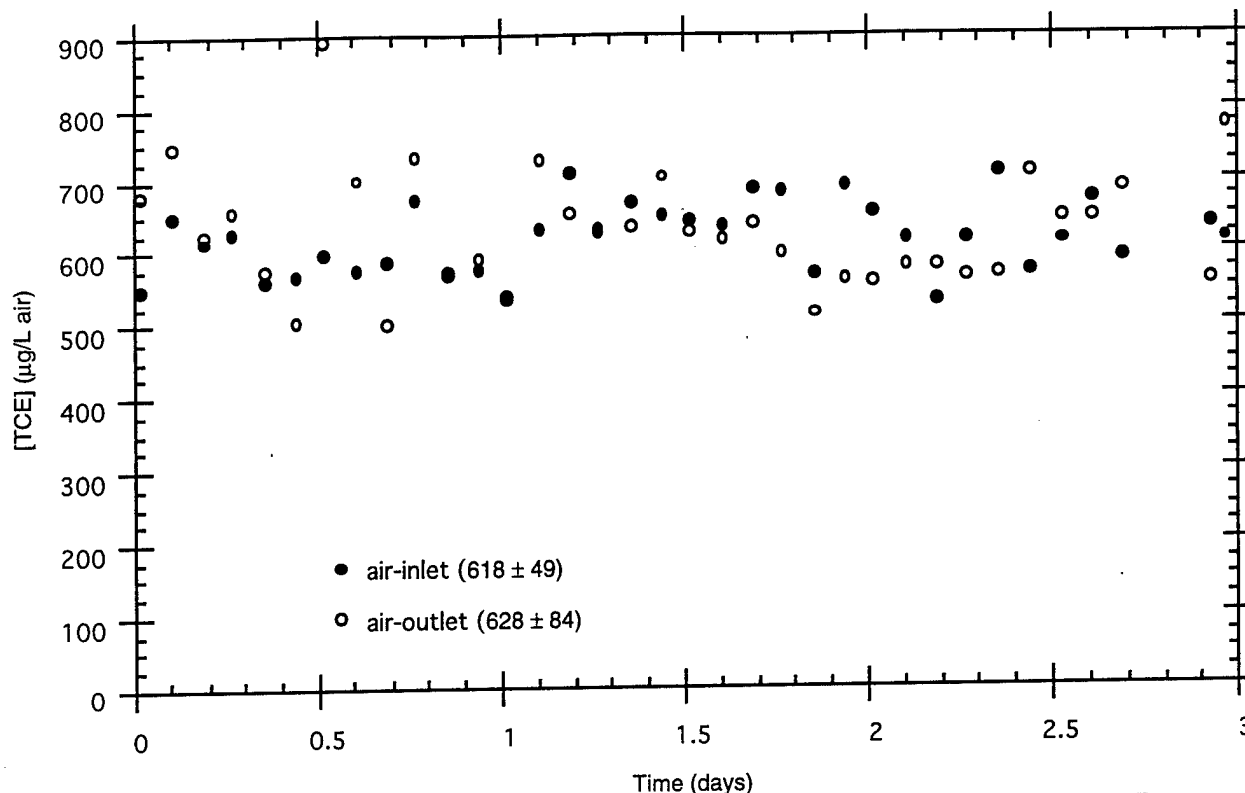


Figure 9: Abiotic Loss Control from the Laboratory Gas-Phase Reactor. TCE concentration was plotted against time of operation for contaminated feed (●) and treated effluent (○).

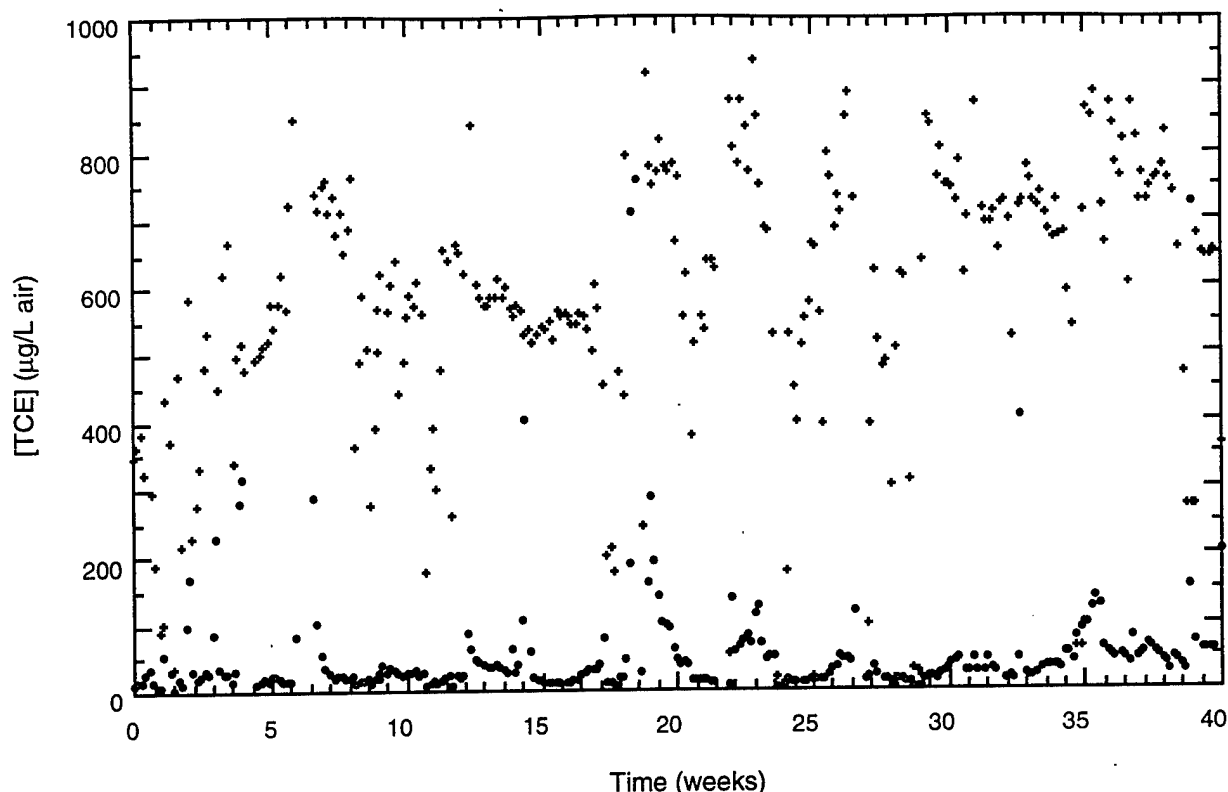


Figure 10: Stable Operation of the Gas-Phase TCE Reactor. Average daily TCE concentration was plotted against time of operation for contaminated feed (+) and treated effluent (O).

F. PRELIMINARY PILOT DEMONSTRATION ACTIVITIES

Equipment for the field demonstration included an FBR, a stripper and a gas-phase bioreactor with ancillary support equipment. The requisite equipment and supplies were shipped to the site, arriving at the end of July, 1994. Over the next month, a number of tasks were completed which included, equipment assembly, installation of electrical service, installation of well pumps and control system, tent erection, and testing of system operation and function. The equipment and trailer were located in a parking lot next to Building 190 and across from Building 181 in the base industrial area (Figure 4). A time line of major events is listed in Table 4.

TABLE 4: FIELD DEMONSTRATION TIME LINE OF MAJOR EVENTS

Date	Day	Event
08/01	-44	equipment arrived and off-loaded at the site
08/15	-30	utility installation complete (electrical, telephone, water, and waste)
08/16	-29	preloading GAC with chemicals begun @ 2 gpm flow rate
08/17	-28	stripper skid assembly complete
08/26	-19	FBR skid assembly and testing complete
08/29	-16	GAC transferred to FBR, preloading continued
09/08	-6	GPR assembly and testing complete
09/07-13	-7	organic chemicals added to FBR to enhance breakthrough
09/10-13	-4	bacterial inoculum added to FBR, switched to 100% recycle
09/14	0	time zero for continuous operation of FBR
09/15	1	bacteria added to GPR, time zero for continuous operation
09/17	3	base air supply interrupted, FBR down for two hours
09/22	8	TCE feed to GPR started @ 10 cfm air flow rate
10/03	19	air flow to GPR reduced to 6 cfm
10/18	34	Air Force personnel tour site
10/19-20	35	GPR system upset and recovery
10/20	36	increase organic load to FBR using spiking system
10/21	37	base air supply interrupted, FBR down for seven hours
11/11	58	end of FBR steady-state operation, start "killed" control
11/15	62	termination of GPR steady-state operation
11/16	63	removed GAC from FBR, start abiotic system loss control
11/17	64	termination of FBR operation
11/22	69	equipment demobilization and decontamination completed

Monitoring wells 1, 6, and 8 (designated MW1, MW6 and MW8, respectively) were hydraulically jetted to enhance yield. Pneumatically actuated submersible pumps were installed which yielded a maximum of 3 gpm per well. Actual flow rates under normal operating conditions averaged 1 to 1.3 gpm per well. MW8 produced low water flow with significant silt. Therefore, MW8 was not used during the demonstration. MW1 and MW6 were manifolded, producing a maximum flow of about 2.5 gpm. Maximum flow rates were limited by both the size of the well and

the hydraulic head loss the pumps had to overcome and apparently not by hydraulic conductivity of the aquifer.

G. FIELD TEST RESULTS FROM OPERATION OF FBR SYSTEM

Field demonstration of the FBR consisted of three phases of process operation: (1) preloading organics and biomass colonization of the GAC; (2) steady-state operation using site water (Day 0 through Day 37) and; (3) steady-state operation spiking additional chemicals into site water to increase loadings (Day 37 through Day 59). The FBR system required about 210 pounds of granular activated carbon (GAC). GAC isotherm studies suggested a minimum of 2 weeks to achieve initial chemical breakthrough for TCE and 1,2-DCB at a contaminated groundwater flow rate of 2 gpm. The GAC used in the FBR was loaded into a 55-gallon drum and contaminated groundwater flow was initiated to maximize the organic load to the GAC as soon as the wells had been installed. Once the FBR was fully assembled and functionally checked, the GAC was transferred to the FBR and preloading operations continued. After 2 weeks of pumping, 2 weeks prior to the projected date for inoculating the FBR, breakthrough was observed. During the week just before inoculation, a total of 1,400 mL TCE, 800 mL 1,2-DCB, 50 mL toluene, and 300 mL unleaded gasoline were added to the FBR feed. These additions led to chemical breakthrough as the GAC adsorption capacity was exceeded (Figure 11). At this time, a total of 32,691 gallons of groundwater had been pumped through the GAC, primarily from MW 1 and MW 6. No efforts were made to inhibit accumulation and growth of bacteria in the reactor during this time period. However, nutrients were not added and pH and DO levels were not controlled. Selected bacterial inoculum, enriched from the laboratory FBR and grown at ENVIROGEN's fermentation facility, were then added to the reactor. This inoculum included 9 liters of BTEX degraders, 7.5 liters of 1,2-DCB degraders and 6 liters of toluene degraders. The FBR was operated in a recycle mode for about 2 days, with no feed flow, to allow the bacteria to attach to the GAC.

Following preloading and inoculation, contaminated groundwater flow was again initiated at approximately 2 gpm, which marked the start of steady-state operation (Day 0). The FBR was operated at an empty bed hydraulic retention time of about 30 minutes. Nutrient addition was initiated and automatic pH and DO controls were activated, set points of 6.8 and 2.0 ppm, respectively. Temperature was recorded but not controlled. Table 5 lists totaled oxygen feed and totaled

groundwater feed flow for operation of the FBR. Table 6 lists selected parameters accumulated by the data logging system on the FBR over the course of steady-state operation.

TABLE 5: FBR PILOT SYSTEM PARAMETERS UNDER STEADY-STATE OPERATION (DAY 0 THROUGH DAY 55).

Operating Parameter	Mean	Standard Deviation	Range
Feed Flow rate, gal/min	2.14	0.34	1.10-2.91
Influent Flow Rate, gal/min	4.51	0.38	4.29-4.96
Fluid Bed Height, ft	12.2	0.4	9.0-12.6
Dissolved Oxygen (DO), mg/L	4.65	2.46	1.90-20.00
pH	6.74	0.30	6.37-7.64
Temperature, °C	23.9	2.0	18.0-28.7
Total Oxygen Feed (scc)	16,830		
Total Groundwater Treated (gal)	210,490		

Values averaged during normal operation and excludes periods when system was completely shut down for maintenance, adjustments, or utility failures.

During the first phase of steady-state operation, effluent quality remained high for BTEX and DCB with a gradual increase in TCE concentrations (Figure 11). Chemical concentrations in the contaminated groundwater feed were very stable. Overall, greater than 90% removal of all chemicals was achieved. Effluent concentrations were less than 20 µg/L for all quantified chemicals except TCE, which averaged 206 µg/L (Table 6). However, removal efficiencies for BTEX were actually higher than reported since calculations were based on minimum detection limits when no integrated peak was observed. FBR performance was exceptional, effectively removing all key chemicals with results fully consistent with the laboratory studies.

TABLE 6: FEED AND EFFLUENT CHEMICAL CONCENTRATIONS DURING STEADY-STATE OPERATION OF THE PILOT FBR (DAY 0 THROUGH DAY 35).

chemical	Feed ($\mu\text{g/L}$)	Effluent ($\mu\text{g/L}$)	% Degraded
benzene	46 ± 30	$<10 \pm 4$	$>78^*$
TCE	$1,445 \pm 173$	206 ± 142	86
toluene	40 ± 46	$<11 \pm 5$	$>73^*$
ethylbenzene	23 ± 17	$<12 \pm 8$	$>46^*$
xylenes	50 ± 29	$<20 \pm 7$	$>40^*$
1,3-DCB	123 ± 10	10 ± 2	92
1,4-DCB	227 ± 20	9 ± 2	96
1,2-DCB	$1,664 \pm 134$	13 ± 5	99

* - Degradation based on detection limit for compounds giving a conservative estimate of performance.

Organic chemical load to the FBR was increased during the next phase of spiked steady-state operation. A chemical feed system was assembled and installed to add a mixture of aromatic and chlorinated chemicals to the contaminated groundwater. This method was chosen because loading could not be increased by increasing either concentration or water flow to the FBR using the existing well system. The feed rate of chemicals from the addition system was incrementally increased starting on Day 36. A mixture of benzene, TCE, toluene, ethylbenzene, xylenes, dichlorobenzenes, and unleaded gasoline was added directly into the contaminated groundwater feed to the FBR at a final rate of about 3 mL/min. Effective concentrations for the chemicals added were increased between 2- and 57-fold thereby increasing total load to the reactor by over sevenfold (Table 7). Chemical concentrations in the feed were more variable than during initial steady-state operations and was attributed to the simplicity of the addition system, the difficulty in controlling flow and incomplete mixing of the neat organic mixture into the water stream. There was some increase in effluent concentrations, although averages were less than 30 $\mu\text{g/L}$ for all quantified chemicals except TCE, which averaged 322 $\mu\text{g/L}$. Effluent samples were taken from the FBR near the end of the second spiked phase of steady-state operation for base neutral extraction and analysis. No chemical intermediates were identifiable in the effluent with

detection limits averaging 0.5 $\mu\text{g/L}$. In general, removal efficiencies were over 97% for all chemicals monitored. Removal efficiencies and FBR performance confirmed results obtained during Phase II SBIR laboratory studies and exceeded performance of the fixed film design tested during the original Phase I SBIR study.

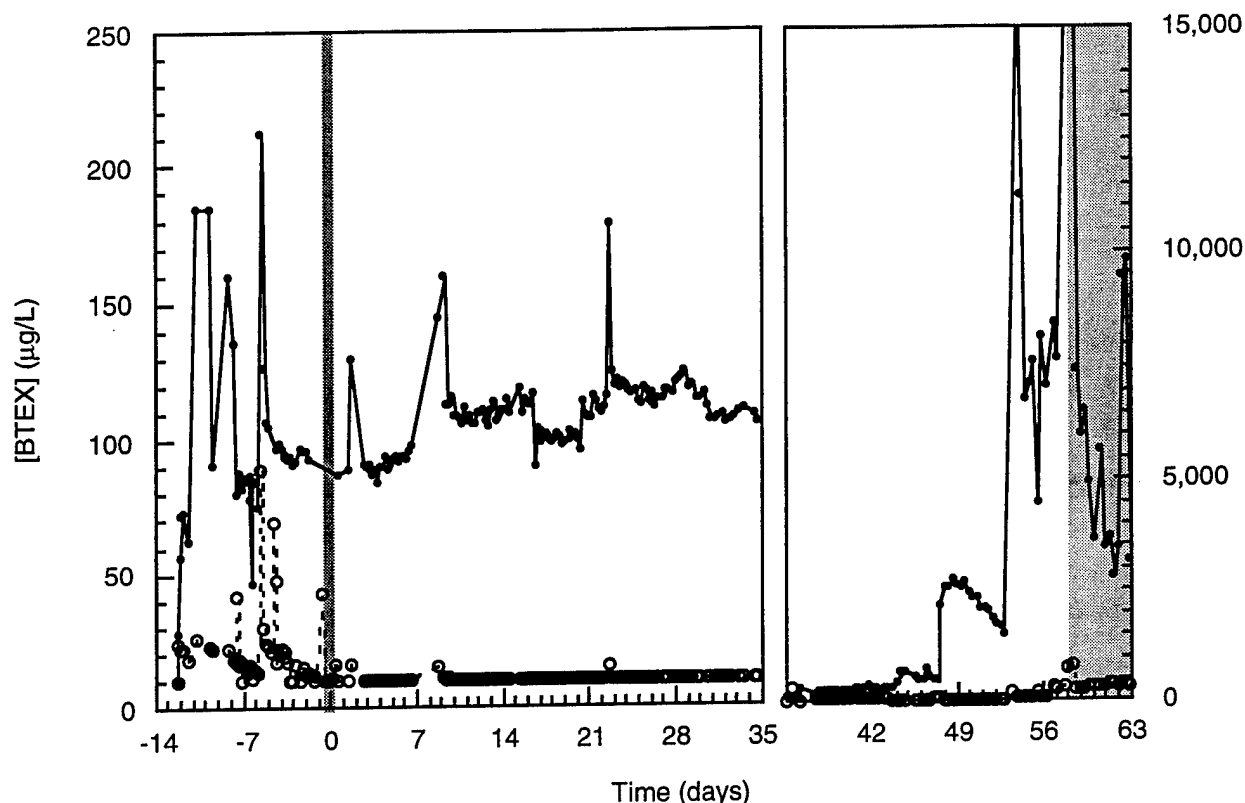


Figure 11A: Performance of the Pilot FBR System. Chemical concentration was plotted against time of operation for contaminated feed (●) and treated effluent (○). The plot is split at day 35 to expand the Y-scale to accommodate higher concentrations during the spiking. The shaded area covering days 57 to 63 cover the time period during the shift to pH 10.

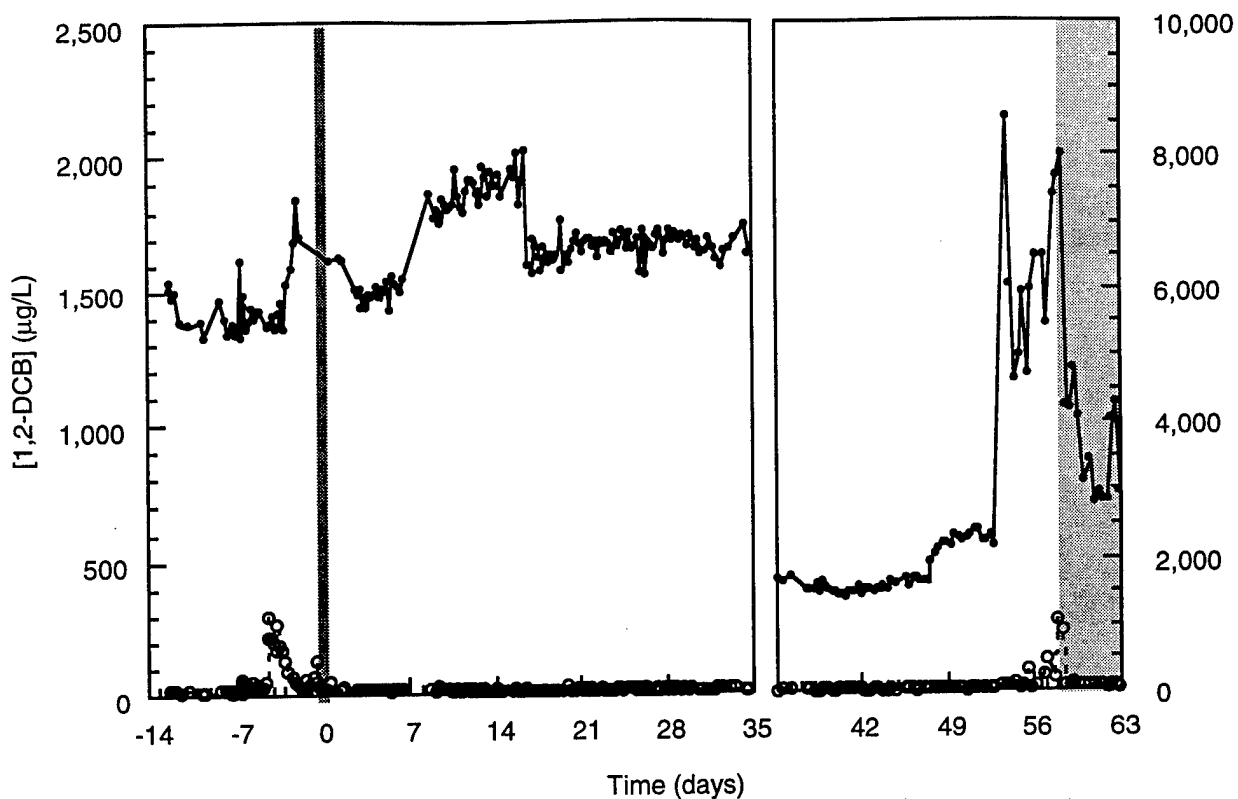


Figure 11B: Performance of the Pilot FBR System. Chemical concentration was plotted against time of operation for contaminated feed (●) and treated effluent (○). The plot is split at day 35 to expand the Y-scale to accommodate higher concentrations during the spiking. The shaded area covering days 57 to 63 cover the time period during the shift to pH 10.

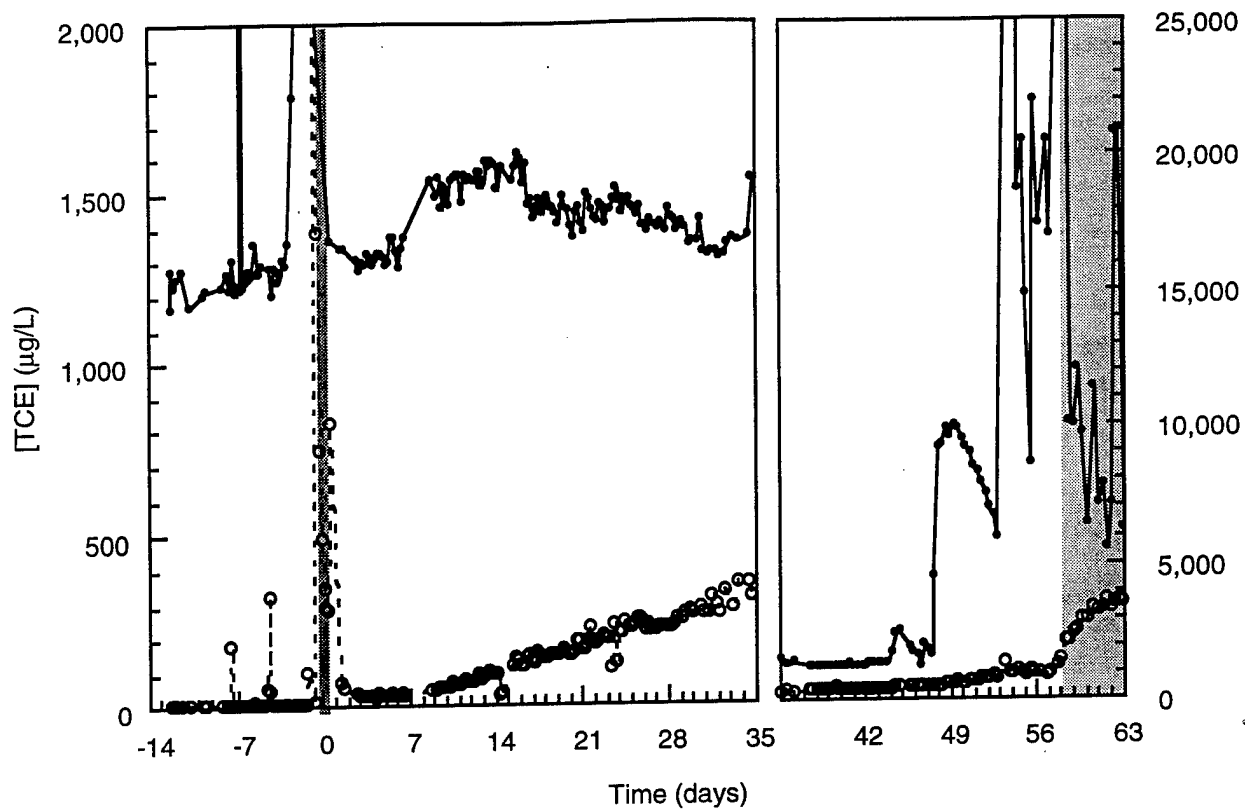


Figure 11C: Performance of the Pilot FBR System. Chemical concentration was plotted against time of operation for contaminated feed (●) and treated effluent (○). The plot is split at day 35 to expand the Y-scale to accommodate higher concentrations during the spiking. The shaded area covering days 57 to 63 cover the time period during the shift to pH 10.

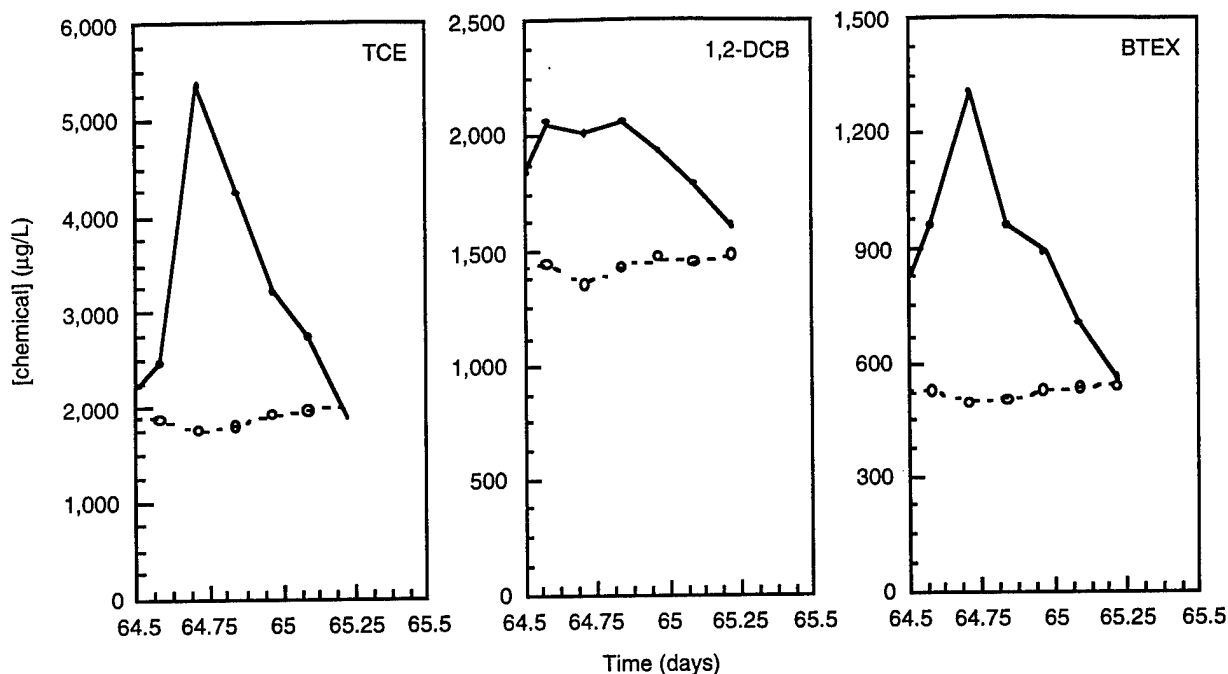


Figure 12: Abiotic loss control from the Pilot FBR System. Chemical concentration was plotted against time of operation for contaminated feed (●) and treated effluent (○) for operation without GAC using contaminated groundwater.

TABLE 7: FEED AND EFFLUENT CHEMICAL CONCENTRATIONS DURING SPIKED STEADY-STATE OPERATION OF THE PILOT FBR (DAY 36 THROUGH DAY 58).

chemical	Feed (µg/L)	Effluent (µg/L)	increased load factor	% Degraded*
benzene	1,544 ± 865	14 ± 22	34	99
TCE	14,911 ± 6,585	322 ± 278	10	98
toluene	2,163 ± 1,007	12 ± 10	54	99
ethylbenzene	1,312 ± 783	14 ± 17	57	99
xylene	710 ± 544	21 ± 20	14	97
1,3-DCB	420 ± 191	10 ± 5	3	97
1,4-DCB	1,435 ± 665	11 ± 10	6	99
1,2-DCB	3,877 ± 1,168	28 ± 92	2	99

* - Degradation based on detection limit for compounds giving a conservative estimate of performance.

At the end of the spiked-feed operating period, the pH set point on the FBR was increased to 10 to inhibit biological activity. Other killing agents were deemed impractical. Although a pH shift to 10 alone does not assure complete elimination of biodegradative activity, it was attempted to establish whether chemical breakthrough would be enhanced. Both BTEX and TCE concentrations in the effluent increased in relationship to feed concentrations which decreased during this time (Figure 11). The effect of pH on adsorption isotherms was not tested. A final control was run where the GAC was removed from the reactor and untreated groundwater was pumped through the FBR to determine abiotic system losses (Figure 12). After about 1 day of operation, feed and effluent concentrations were essentially equal. The cumulative GAC loadings for these chemicals were calculated from concentration and flow data collected weekly during the field demonstration (Figure 13). Cumulative carbon loading increased each week with time zero closest to $Y=0$ and each subsequent week of operation higher along the Y-axis. The boxed crosses show a shift in equilibrium during the spiking operation. The last point demonstrates a rapid transition to the isotherm when the system was shifted to pH 10 at the very end of the study. These results indicate that the FBR was effectively biodegrading all of the chemicals removed during treatment. Partial or complete inactivation of biological activity led to accelerated chemical breakthrough.

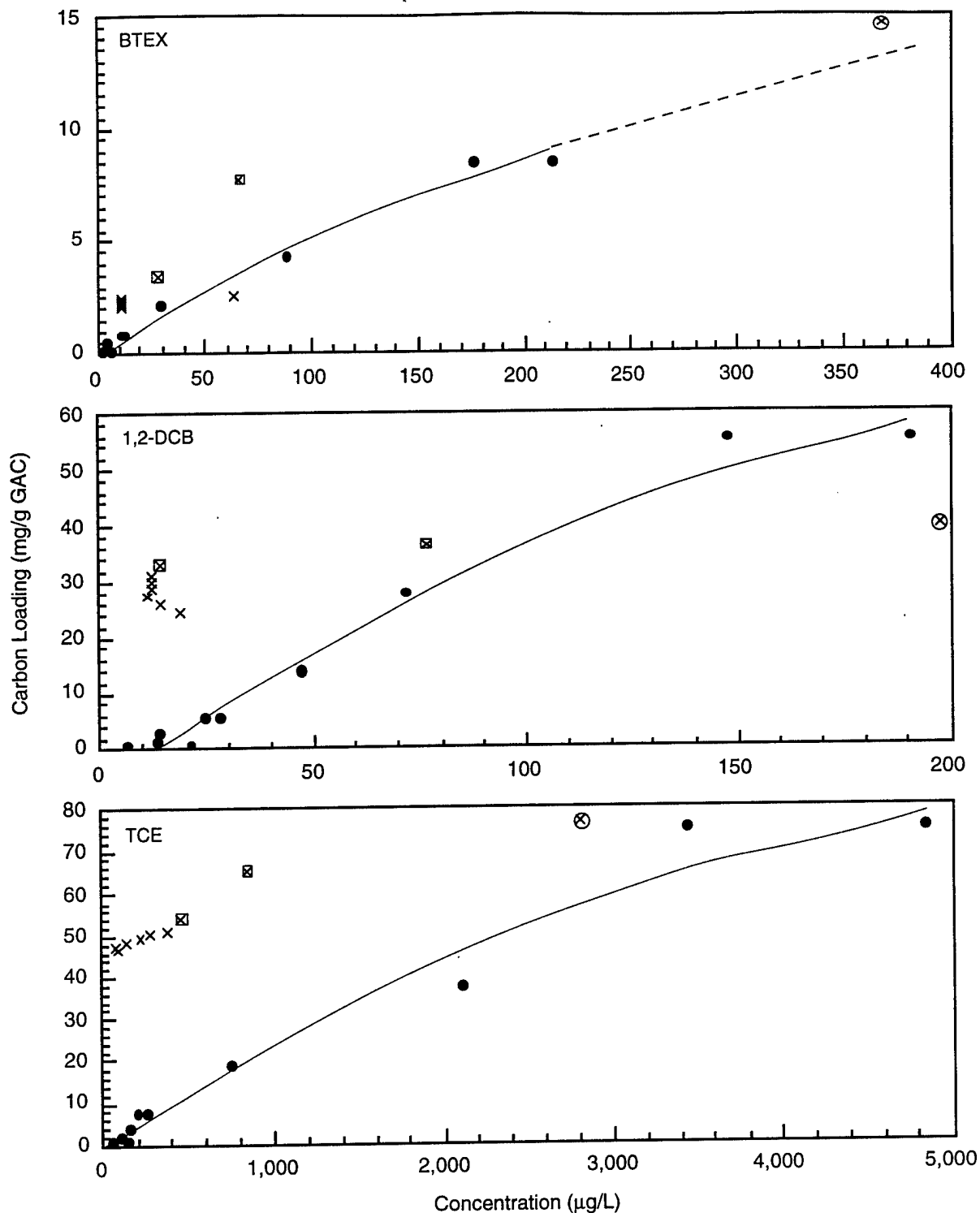


Figure 13: Organic Loading to FBR GAC During Pilot Test. Cumulative weekly carbon loading was plotted against effluent chemical concentration for BTEX (A), 1,2-DCB (B) and TCE (C). Also plotted are the GAC isotherms from Figure 7. The different plot symbols represent the different phases of operation: (x) initial steady state, (⊠) spiked steady state and (⊗) killed.

Samples of the GAC bed material were removed from the reactor and shipped back to the ENVIROGEN prior to the final pH shift. An aliquot of this sample was vigorously shaken to remove active biomass from the GAC and the biomass fraction was transferred to serum bottles. A standard bottle assay was performed to assess degradative capabilities using the mixture of chemicals listed in Table 8. Initial concentrations for all samples were essentially those of the "killed" control. Serum bottles with intact septa typically demonstrated less than 5% loss over a 48 hour time period. All chemicals analyzed were degraded except TCE (Table 8). These data alone do not support the conclusion that TCE was actively biodegraded in the FBR. However, other observations, such as those outlined above, support this conclusion. The aerobic co-metabolic degradation of TCE may require unique conditions found in the FBR under normal operation that were not duplicated in the bottle assay. Biomass recovered from the pilot FBR, however, did retain biocatalytic capabilities directly confirming that the FBR system contained bacteria capable of degrading all targeted chemicals which support growth and metabolism.

TABLE 8: DEGRADATIVE ACTIVITY OF BIOMASS REMOVED FROM PILOT FBR.

chemical	"killed" ($\mu\text{g/L}$)	"live" ($\mu\text{g/L}$)	% Degraded
benzene	142	26	82%
TCE	2290	2714	-19%
toluene	83	>1	99% ^a
ethylbenzene	54	>1	99% ^a
<i>m,p</i> -xylenes	23	>1	98% ^a
<i>o</i> -xylenes	27	>1	97% ^a
1,3-DCB	302	9	97%
1,4-DCB	148	6	96%
1,2-DCB	3529	15	100%

All bottles had oxygen enriched headspace and incubated at 22°C for 48 hours, pH 7.4. Killed controls had sodium azide added to a final concentration of 0.1%. Purge and trap GC/FID analysis was performed on 2.0 mL of liquid. Each data point represents two bottles, each analyzed in duplicate. A - Calculated based on detection limit.

The bed material removed from the FBR was also subjected to methanol extraction to quantify the amount of key organic chemicals bound to the GAC (Table 9). As demonstrated earlier, the methanol extraction method was able to extract essentially all of the TCE from virgin GAC. Chemical recoveries from the GAC were consistent with the isotherms generated using virgin carbon. The amount of chemicals bound to the carbon were greatest at the bottom of the reactor. This was consistent with the pseudo-plug flow operation of the FBR system in which the highest concentration of chemicals in the water was greatest at the bottom of the reactor. The total organic load to the carbon was less than the maximal holding capacity due to isotherm effects at the chemical concentrations in the feed and effluent streams. Maximal loading capacities are only achieved when the carbon can be saturated, which clearly was not achievable under these operating conditions. A total mass balance was determined for operation of the pilot FBR system (Table 10). Loading calculations were based on weekly averages for feed and effluent chemical concentrations and totaled liquid flow to the system. The loading calculations also included the chemicals added to enhance breakthrough (adjusted for losses to the effluent). GAC loading was based on 95 kg of activated carbon in the reactor and the amount of organic sorbed to carbon at the bottom of the reactor from Table 8. Overall, a total of 83% of the TCE, 93% of the 1,2-DCB, and 67% of the BTEX were destroyed during FBR operation using the conservative estimate of bound chemicals. These results clearly demonstrated a significant loss of chemical as a result of biodegradative activity beyond the binding capacity of the GAC in the reactor.

TABLE 9: METHANOL EXTRACTABLE ORGANIC CHEMICALS REMOVED FROM PILOT FBR.

Chemical	bottom sample (mg/g GAC)	middle sample (mg/g GAC)	top sample (mg/g GAC)
TCE	9.61	<0.01	<0.01
1,2-DCB	1.95	0.20	0.31
BTEX	5.16	0.84	0.69

A 1 g GAC sample was extracted with 9 mL of methanol. The methanol phase was injected onto a GC and the amount of chemical quantified using an external standard. The bottom sample represents the average of 4 separate extractions and analysis.

TABLE 10: CHEMICAL MASS BALANCE FOR KEY CONTAMINANTS DURING OPERATION OF THE PILOT FBR.

Chemical	total input in feed (g)	total output in effluent (g)	net load to reactor (g)	total bound to GAC (g)	net destroyed (g)
TCE	6041	518	5523	912	4611
1,2-DCB	2830	92	2738	185	2553
BTEX	1536	65	1471	490	981

H. FIELD TEST RESULTS FROM OPERATION OF GPR SYSTEM

As part of the GPR functional testing, an abiotic loss control was performed. The reactor was filled with water and TCE-contaminated air was introduced at a flow rate of 10 cfm. TCE concentrations in the feed and effluent air streams were monitored in the absence of degradative activity (Figure 14). Over the 24 hours of the test, the average TCE concentration in the feed and effluent streams was 522 ± 64 and 492 ± 63 $\mu\text{g/L}$ air respectively. The GPR system demonstrated 94% recovery under standard operating conditions.

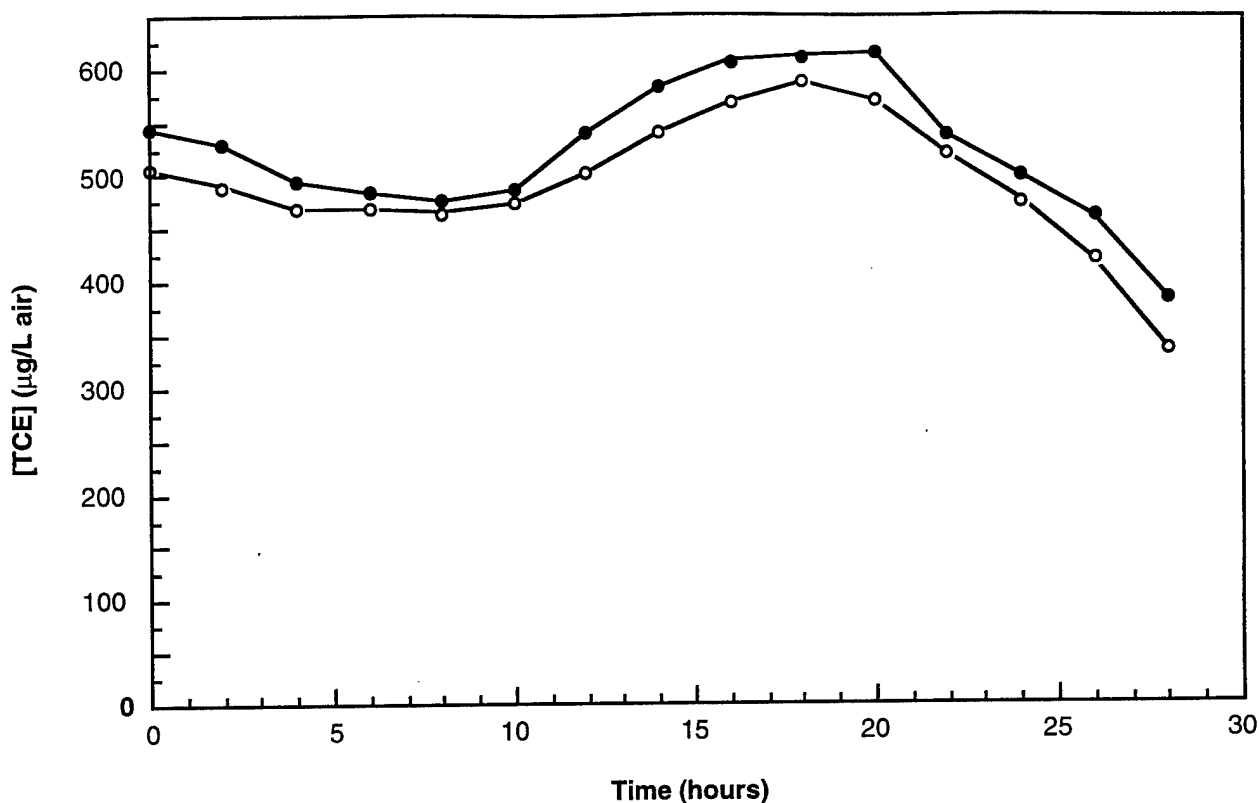


Figure 14: Abiotic Loss Control for Operation of the Pilot GPR. TCE concentration was plotted against time of operation for contaminated feed (●) and treated effluent (○).

The GPR was then prepared for normal operation with active biomass. An inoculum of TCE degradative bacteria was grown with phenol as the sole carbon source at ENVIROGEN's fermentation facilities. These organisms were added to the GPR giving a cell density of 0.2 mg/mL protein. The reactor was then operated in batch mode, without TCE, to allow for biomass growth. The system was switched to flow-through operation and TCE addition was initiated when the biomass level reached 0.35 mg/mL several days later. Since the FBR system was not only effectively treating BTEX and DCB, but also TCE, additional TCE was required to adequately test GPR performance. Therefore, a chemical addition system was installed to deliver TCE into the air entering the GPR from the air stripper. Initially, TCE concentrations were higher than desired and modifications were made to the spiking system to improve control. Over the course of steady-state operation, average feed and effluent concentrations for TCE were 371, and 80 µg/L air respectively (Figure 15). Under normal operation, phenol concentrations

were less than the detection limit of 0.1 ppm. Selected operating parameters are listed in Table 11. On Day 37 the bacteria in the reactor lost activity against TCE and phenol. At this time, phenol concentrations in the reactor exceeded 100 ppm. This deviation in activity was traced to a mechanical issue with the pH control and delivery system. An excursion in pH led to an apparent cell lysis and foaming event. Consequently, about 75% of the biomass was lost which significantly lowered the volumetric performance capacity of the reactor. The pH control issue was rectified and the reactor was temporarily switched to batch operation to allow for recovery. Within 24 hours, biomass levels doubled from 0.4 to 0.8 mg/mL protein at which time the TCE feed was re-initiated and normal operation resumed. For the time period leading up to system upset, the GPR degraded an average of 85 % of the TCE. Following recovery, performance dropped to an average of 70 %. The average TCE removal efficiency was 74% for the entire demonstration. Performance efficiencies were lower during pilot GPR operation than during laboratory system operation because the pilot GPR was operated at a lower biomass levels. Performance can be optimized by increasing the biomass levels in the reactor. The pilot demonstration confirmed that the GPR has enhanced stability and is capable of operating for extended periods of time under field conditions. This was a major breakthrough in development of this innovative technology.

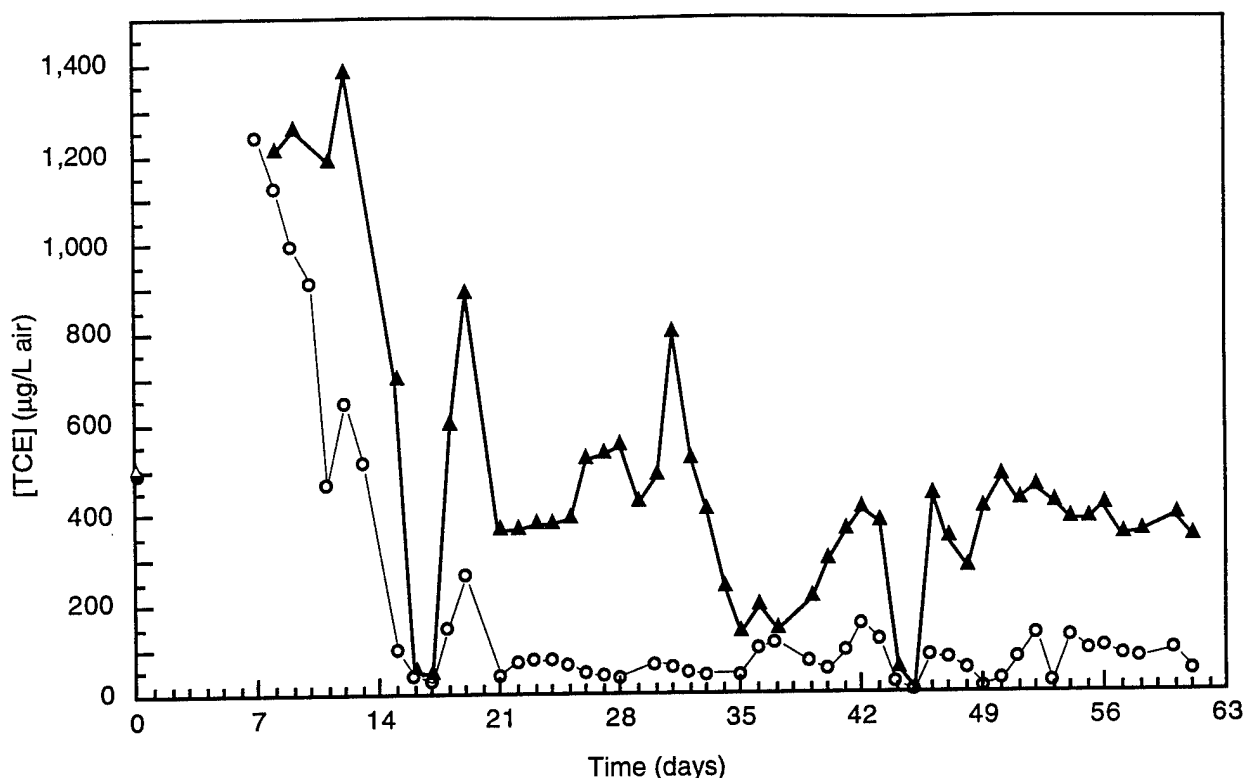


Figure 15: Steady-State Performance of the Pilot GPR. Average daily TCE concentration was plotted against time of operation for contaminated feed (\blacktriangle) and treated effluent (\circ). The average feed and effluent TCE concentrations for the control are plotted at time zero from Figure 14.

TABLE 11: GPR PILOT SYSTEM PARAMETERS UNDER STEADY-STATE OPERATION(DAY 0 THROUGH DAY 60).

Operating Parameter	Mean	Standard Deviation	Range
Air Flow rate, cfm	7		4-10
pH	6.8	0.2	6.5-8.0
Temperature, °C	26	2	
[biomass], mg/mL protein	1.2	0.5	0.3-2.3
[TCE _{influent}], µg/L air	371	173	3 - 1771
[TCE _{effluent}], µg/L air	80	55	0 - 665
% Degraded	74	24	0 - 100

I. ECONOMIC EVALUATION FOR FULL-SCALE SYSTEM

An economic evaluation was performed based on key parameters expected to represent those typically found at contaminated sites. The standard contaminated groundwater characteristics used for analysis were a feed flow rate of 100 gpm, containing 15 ppm BTEX (ratio of 7.5:4:1.5:2) with 1 ppm TCE. Capital and operating costs were developed for ENVIROGEN's FBR system, wet carbon adsorption, air stripping/dry carbon adsorption, UV/peroxidation and air stripping/PURUS A-3000 using this set of flow rates and concentrations (Table 12). All cost estimates include installation of the complete system on customer supplied foundations. All cost estimates exclude: (1) routing of groundwater to the system; (2) routing treated effluent from the system; (3) start-up; (4) field supervision; (5) equipment freight; (6) taxes and; (7) additional, site specific pre- or post-treatment equipment requirements. An estimated cost of \$0.07/kwh was used for power requirement calculations and a rate of \$50/hr was used for labor requirements calculations. Carbon replacement costs were set at \$2.00/pound for both wet and dry carbon adsorption options which include replacement carbon and extras such as vacuuming, shipping, removal and disposal of spent carbon. For the ENVIROGEN FBR, carbon replacement was also assumed to be \$2.00/pound for fresh carbon, even though no extra services were required. The ENVIROGEN FBR carbon attrition rate was assumed to be 5% (approximately 320 pounds/year). Estimates of carbon usage for the air stripping with carbon adsorption were obtained from two vendors. These estimates were 112 and 136 pounds/day with internal ENVIROGEN estimates at 132 pounds/day. For the ENVIROGEN FBR system, the desired effluent quality can be met using a 5 ft diameter by 11 ft tall fluidized bed bioreactor (Figure 16). The 5 ft ENVIROGEN FBR system with ancillary equipment would occupy an area of less than 240 ft². The ENVIROGEN FBR system capital cost used was \$200,000 with an operating cost of \$17,520/year including power, nutrients, carbon replacement due to attrition, manpower and maintenance costs. As shown in Figure 17, the break even point for the ENVIROGEN FBR is 1.6 years as compared to the best alternative technology, air stripping/carbon adsorption. This payback reflects an \$82,000 savings in yearly operating and maintenance costs for the ENVIROGEN FBR system compared to air stripping/carbon adsorption. The cumulative total cost savings (operating, maintenance and capital) for a 10-year project would be \$690,000.

TABLE 12: COMPARATIVE ECONOMICS FOR FBR AND ALTERNATIVE TREATMENT TECHNOLOGIES.

System	Area Requirement	Capital Cost	Annual Operating Cost
ENVIROGEN Fluidized Bed Bioreactor + spare effluent pump	240 ft ² 10 ft x 24 ft	\$200,000	\$5,800 power \$1,320 carbon/nutrients \$10,400 labor/maintenance ¹ \$17,520 total
UV-peroxidation + bag filters	187 ft ² 11 ft x 17 ft	\$191,250	\$84,050 power \$34,050 chemicals \$21,200 labor/maintenance \$139,300 total
carbon adsorption (wet) + dual bag filters + spare effluent pump	576 ft ² 24 ft x 24 ft	\$80,000	\$1,372 power \$165,628 carbon replacement ² --- labor/maintenance ³ \$167,000 total
air stripping/ carbon adsorption (dry) + dual bag filters		\$70,000	\$8,920 power ⁴ \$90,520 carbon replacement ⁵ --- labor/maintenance ³ \$99,440 total
air stripping/PURUS A-3000 adsorption		\$260,000	\$54,000 includes nitrogen, power, maintenance, and air stripper ⁶

- (1) Assumes 8 hour operator attention every two weeks at \$50/hr using local labor.
- (2) Total carbon, 14,400 pounds with 50% replacement required once per month.
- (3) Labor and maintenance costs are included in the \$2.00/pound carbon replacement costs.
- (4) Includes a 7.5 HP pump for liquid flow and a 9 kw in-line heater to reduce relative humidity to 50%.
- (5) Assumes 124 pounds/day usage at \$2.00/pound (0.155 pounds hydrocarbon/pound carbon).
- (6) Includes \$36,000 annual operating costs from PURUS article, Research Magazine, April 1994 and \$18,000 of power, operating and maintenance costs associated with the air stripper.

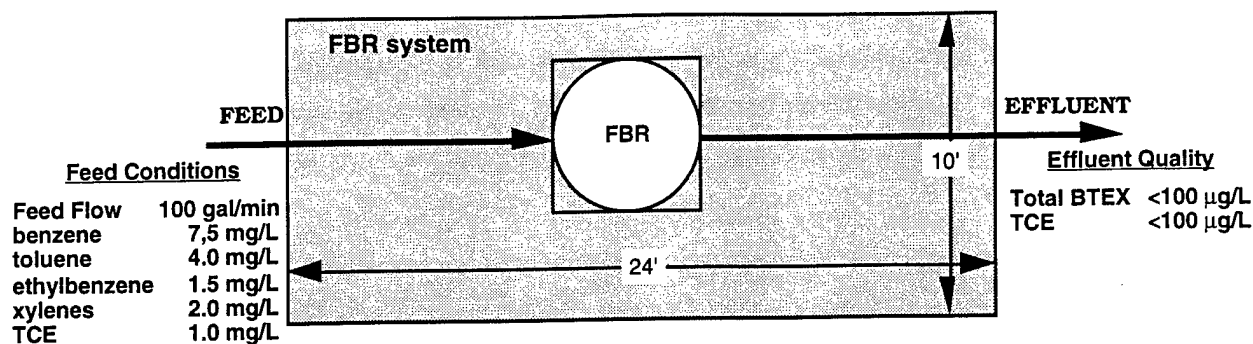


Figure 16: Process Flow Diagram for Standard FBR Systems.

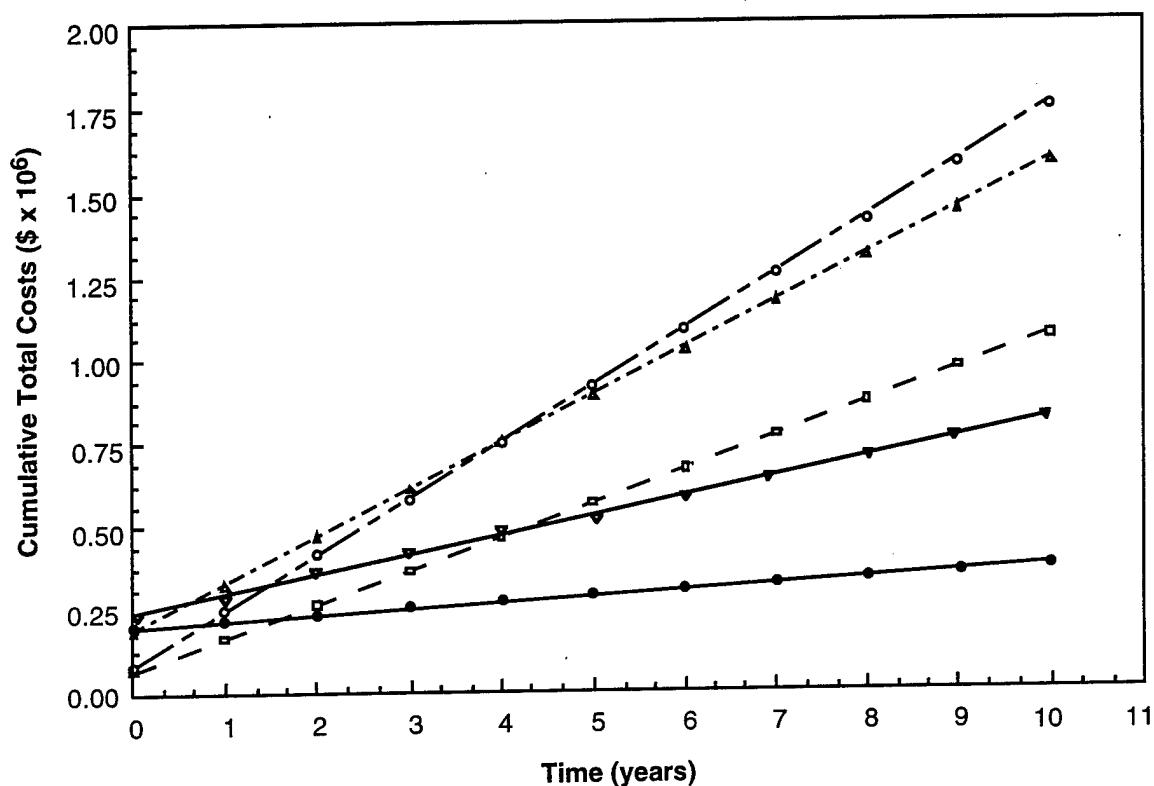


Figure 17: Life Cycle Costs of FBR and Carbon Adsorption Systems. The FBR system (●) is compared to UV/peroxidation (Δ), air stripping with dry carbon adsorption (□), and wet carbon adsorption (○). (▽) Air stripping with PURUS A-3000 system. Costs represent current dollars with no amortization period or interest rate factor.

An economic evaluation was also performed for the GPR system based on key parameters expected to represent those typically found at contaminated sites during soil vapor extraction (SVE) operations. Assuming an air flow rate of 300 cfm

and TCE concentrations in the air of either 100 or 300 ppmv, an 11 ft diameter, 7,500 gal GPR is required to achieve the desired treatment level (Figure 18). System costs were estimated at \$125,000 \pm 15% installed on customer supplied foundations. Operating costs were estimated at \$25,000/year including power (@ \$0.07/kwh), nutrients, manpower and maintenance costs.

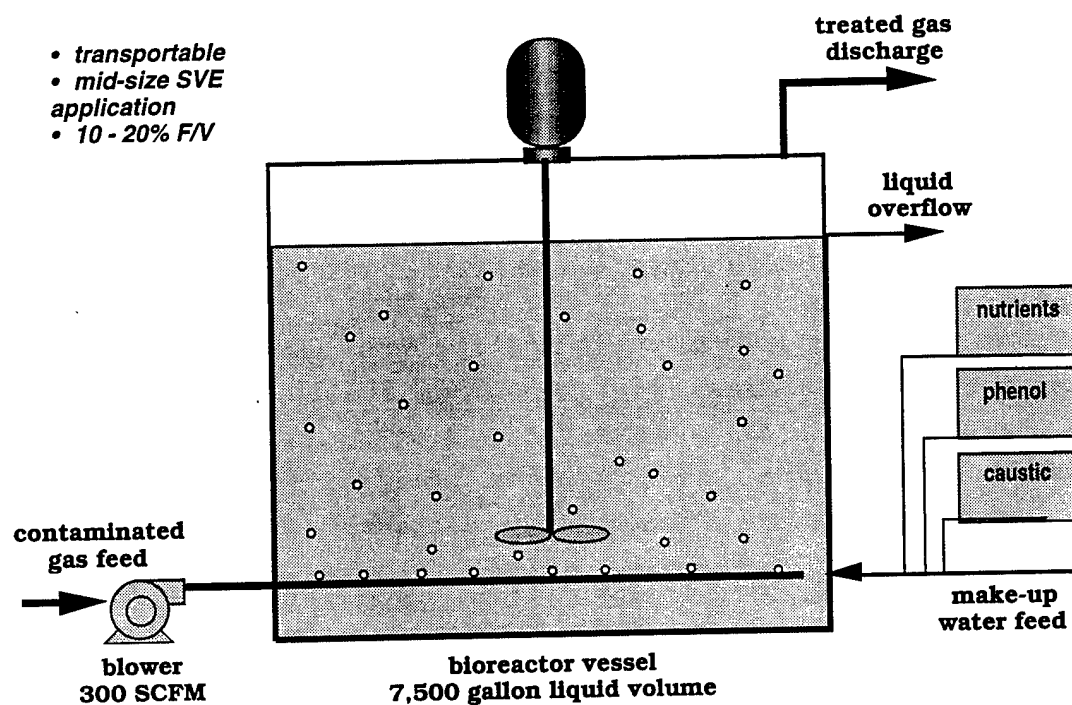


Figure 18: Process Flow Diagram for Standard GPR.

Comparable capital and operating costs were developed for carbon adsorption using the same set of flow rates and concentrations. Carbon consumption was based on theoretical isotherm data and changed significantly for the two concentrations of TCE used (Figure 19). A treatability study would be required to determine actual consumption rates. Capital costs were estimated at \$10,000 \pm 15%. System costs included complete carbon adsorption system installed on customer supplied foundations. Costs excluded installation of SVE system, routing air to and from the carbon adsorbers and any start-up and field supervision services. The annual operating costs were estimated to range from \$50,000 to \$250,000 depending on TCE concentrations. Carbon replacement service costs may vary depending on site location.

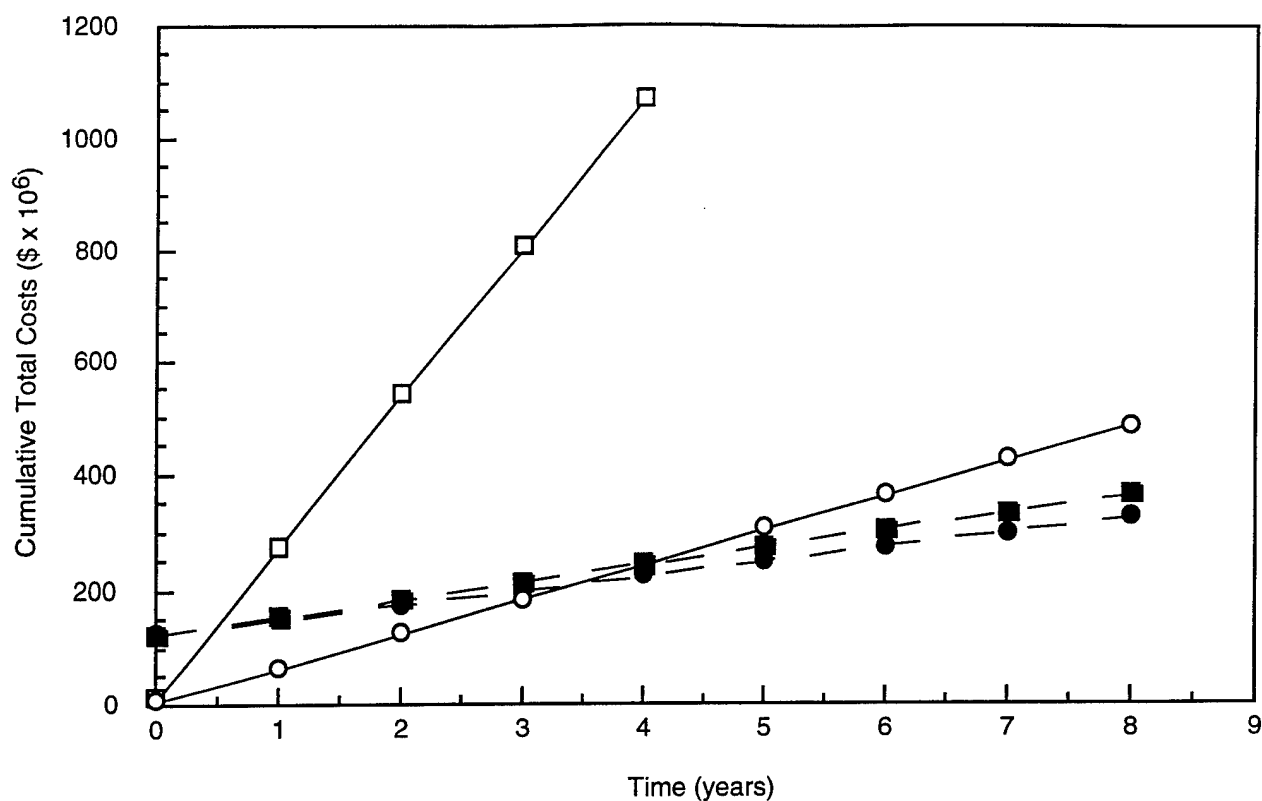


Figure 19: Life Cycle Costs of GPR and Carbon Adsorption Systems. Costs represent current dollars with no amortization period or interest rate factor. The four curves are for the GPR at 100 ppmv (●) and 300 ppmv (■) and for carbon adsorption at 100 ppmv (○) and 300 ppmv (□).

SECTION V: CONCLUSIONS

The dual phase bioreactor system performed well during the demonstration at Robins Air Force Base. The FBR effectively removed >97% of the 1,2-DCB and >95% of the BTEX from the water over the time period including preloading, steady state and spiked phases of operation. During this same time period, aqueous TCE concentrations were reduced by an average of 88% with a total mass balance demonstrating greater than 84% destruction beyond carbon adsorption in the FBR. Performance of the FBR exceeded expectations, demonstrating effective removal of the BTEX and DCB but also significant removal of TCE.

Due to the high performance of the FBR the vapor entering the GPR for treatment had to be spiked with TCE. TCE was reduced by an average of 75% in the GPR. This performance can be improved to over 90% by increasing the biomass concentrations in the reactor as demonstrated with the laboratory systems. The major issue with the GPR at the beginning of this project concerned operational stability. This issue was successfully overcome with 10 months of continuous operation using the laboratory system and 2 months of continuous operation in the field. In essence, two independent field demonstrations were successfully performed under this contract. Over 210,000 gallons of contaminated groundwater were effectively treated during the demonstration. All hazardous chemicals were treated to concentrations near or below drinking water standards.

Economic evaluations of both fluidized bed bioreactor technology and gas phase bioreactor systems to alternative treatment options, including carbon adsorption, suggests a significant cost savings over the life of a typical project. Though capital costs for either FBR or GPR are higher than for carbon adsorption, operating costs are dramatically lower leading to a 1 to 2 year payback. Biological treatment is a destructive technology, eliminating the hazard, whereas carbon adsorption would require additional treatment or containment of the contaminated, used carbon. If chemical concentrations are higher than the assumptions used in the estimates, operating costs for carbon adsorption will increase, whereas FBR and GPR operating costs will not change significantly. Biological treatment provides a economic, destructive technology for remediating contaminated air or water.

SECTION VI: RECOMMENDATIONS

Selection of an appropriate remediation system depends on the specifics of the contaminated site and treatment requirements. ENVIROGEN's proprietary bioreactor systems are available for treatment of contaminated groundwater. Specification of each system option will depend on chemical concentration and composition, groundwater flow rates, and effluent treatment criteria at individual sites. Depending on the composition and concentration of the components, some minor modifications to system operation, or chemical amendments may be required to optimize TCE removal efficiencies at different sites. Where soil vapor extraction is appropriate for remediating unsaturated soils or where air stripping operations are in place, the GPR system will provide a cost effective innovative treatment technology for reducing air emissions of TCE. The innovative technology demonstrated during this project is currently available for installation and operation for remediation of contaminated water, either surface or groundwater. Both reactor types can be installed at contaminated sites and operated as part of full-scale remediation operations.

SECTION VII: REFERENCES

1. Vogel, T.M. and P. McCarty. 1985. "Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride and carbon dioxide under methanogenic conditions," Appl. Env. Microbiol. 49:1080-1083.
2. 1987. Health Advisories for 25 Organics: Benzene, U.S. EPA PB 87-235578, p 19.
3. Ensley, B.D., 1991. "Biochemical Diversity of Trichloroethylene Metabolism," Annu. Rev. Microbiol. 45:283-299.
4. Arciero, D., T. Vannelli, M. Logan, and A.B. Hooper. 1989. "Degradation of trichloroethylene by the ammonia-oxidizing bacterium *Nitrosomonas europaea*," Biochem. Biophys. Res. Comm. 159:640-643.
5. Folsom, B.R. and P.J. Chapman. 1990. "Phenol and trichloroethylene degradation by *Pseudomonas cepacia* G4: Kinetics and interactions between substrates," Appl. Env. Microbiol. 57:1602-1608.
6. Little, C.D., A.V. Palumbo, S.E. Herbes, M.E. Lidstrom, R.L. Tyndall and P.J. Gilmer. 1988. "Trichloroethylene biodegradation by a methane-oxidizing bacterium. Appl. Env. Microbiol. 54:951-956.
7. Nelson, M.J.K., S.O. Montgomery, E.J. O'Neill, and P.H. Pritchard. 1986. Aerobic metabolism of trichloroethylene by a bacterial isolate," Appl. Env. Microbiol. 52:383-384.
8. Nelson, M.J.K., S.O. Montgomery, W.R. Mahaffey, and P.H. Pritchard. 1987. "Biodegradation of trichloroethylene and involvement of an aromatic biodegradation pathway," Appl. Environ. Microbiol. 53:949-954.
9. Oldenhuis, R., R.L.J.M. Vink, D.B. Janssen, and B. Witholt. 1989. "Degradation of chlorinated aliphatic hydrocarbons by *Methylosinus trichosporium* OB3b expressing soluble methane monooxygenase," Appl. Environ. Microbiol. 55:2819-2826.
10. Shields, M.S., S.O. Montgomery, P.J. Chapman, S.M. Cuskey, and P.H. Pritchard. 1989. "Novel pathway of toluene catabolism in the trichloroethylene degrading bacterium, G4," Appl. Environ. Microbiol. 55:1624-1629.
11. Tsien, H.C., G.A. Brusseau, R.S. Hanson, and L.P. Wackett. 1989. "Biodegradation of trichloroethylene by *Methylosinus trichosporium* OB3b," Appl. Environ. Microbiol. 55:3155-3161.
12. Wackett, L.P. and D.T. Gibson. 1988. "Degradation of trichloroethylene by toluene dioxygenase in whole-cell studies with *Pseudomonas putida* F1," Appl. Environ. Microbiol. 54:1703-1708.
13. Wilson, J.T. and B.H. Wilson. 1985. "Biotransformation of trichloroethylene in soil," Appl. Environ. Microbiol. 49:242-243.

14. Zylstra, G.J., L.P. Wackett, and D.T. Gibson. 1989. "Trichloroethylene degradation by *Eschericia coli* containing the cloned *Pseudomonas putida* F1 toluene dioxygenase genes," Appl. Environ. Microbiol. 55:3162-3166.
15. Allen, B.R., K.R. Anderson and R.A. Ashworth. 1989. "Biological treatment of trichloroethylene contaminated groundwater," In G.Lewandowski, P. Armenante and B. Baltzis. (eds.) Biotechnology Applications in Hazardous Waste Treatment. Engineering Foundation, New York, NY.
16. Coyle, C.G., Parkin, G.F., and Gibson, D.T., 1993, "Aerobic, phenol-induced TCE degradation in completely mixed, continuous-culture reactors," Biodegradation 4:59-60.
17. Ensley, B.D. and Kurisko, P.R., 1994, "A gas lift bioreactor for removal of contaminants from the vapor phase," Appl. Environ. Microbiol. 60:285-290.
18. Folsom, B.R. and P.J. Chapman. 1991. "Performance characterization of a model bioreactor for the biodegradation of trichloroethylene by *Pseudomonas cepacia* Strain G4," Appl. Environ. Microbiol. 57:1602-1608.
19. Landa, A.S., Sipkema, E.M., Weijma, J., Beenackers, A.A.C.M., Dolfing, J., and Janssen, D.B., 1994, "Cometabolic degradation of trichloroethylene by *Pseudomonas cepacia* Strain G4 in a chemostat with toluene as the primary substrate," Appl. Environ. Microbiol. 60:3368-3374.
20. Phelps, T.J., J.J. Niedzielski, R. M. Schram, S E. Herbes, and D.C. White. 1990. "Biodegradation of trichloroethylene in continuous recycle expanded-bed bioreactors," Appl. Environ. Microbiol. 56:1702-1709.
21. Strandberg, G.W., T.L. Donaldson, and L.L. Farr. 1989. "Degradation of trichloroethylene and trans-1,2-dichloroethylene by a methanotrophic consortium in a fixed-film, packed be bioreactor," Environ. Sci. Technol. 23:1422-1424.
22. Wilson, J.T., J.M. Henson, M.D. Piwoni, B.H. Wilson and P. Banerjee. 1988. Biodegradation and sorption of organic solvents and hydrocarbon fuel constituents in subsurface environments. ESL-TR-87-52, Engineering and Services Laboratory, Tyndall AFB, FL, 1988.
23. Biodegradation of chlorinated hydrocarbons in a vapor phase reactor. 1991. Contract No. 02112407. Research and Development Program Coordination Office, Chemical Technology Division, Argonne National Laboratory, Argonne, IL.
24. Folsom, B.R., Liquid-Phase Bioreactor for Degradation of Trichloroethylene and Benzene. ESL-TR-92-02, Engineering and Services Laboratory, Headquarters Air Force Engineering and Services Center, Tyndall AFB FL, April 1993.